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SUBCHAPTER D—DRUGS FOR HUMAN USE

PART 300—GENERAL

Subpart A [Reserved]

Subpart B—Combination Drugs

Sec.
300.50 Fixed-combination prescription drugs for humans.

Subpart C—Substances Generally Prohibited From Drugs

300.100 Chlorofluorocarbon propellants.

Subpart A [Reserved]

Subpart B—Combination Drugs

AUTHORITY: 21 U.S.C. 331, 351, 352, 355, 357, 360b, 361, 371.

§ 300.50 Fixed-combination prescription drugs for humans.

The Food and Drug Administration's policy in administering the new-drug, antibiotic, and other regulatory provisions of the Federal Food, Drug, and Cosmetic Act regarding fixed combination dosage form prescription drugs for humans is as follows:

(a) Two or more drugs may be combined in a single dosage form when each component makes a contribution to the claimed effects and the dosage of each component (amount, frequency, duration) is such that the combination is safe and effective for a significant patient population requiring such concurrent therapy as defined in the labeling for the drug. Special cases of this general rule are where a component is added:

(1) To enhance the safety or effectiveness of the principal active component; and

(2) To minimize the potential for abuse of the principal active component.

(b) If a combination drug presently the subject of an approved new-drug application or antibiotic monograph has not been recognized as effective by the Commissioner of Food and Drugs based on his evaluation of the appropriate National Academy of Sciences-National Research Council panel report, or if substantial evidence of effectiveness has not otherwise been presented for it, then formulation, labeling, or dosage changes may be proposed and any resulting formulation may meet the appropriate criteria listed in paragraph (a) of this section.

(c) A fixed-combination prescription drug for humans that has been determined to be effective for labeled indications by the Food and Drug Administration, based on evaluation of the NAS–NRC report on the combination, is considered to be in compliance with the requirements of this section.

[40 FR 13496, Mar. 27, 1975]

Subpart C—Substances Generally Prohibited From Drugs

§ 300.100 Chlorofluorocarbon propellants.

The use of chlorofluorocarbons in human drugs as propellants in self-pressurized containers is generally prohibited except as provided by § 2.125 of this chapter.

[43 FR 11317, Mar. 17, 1978]

PART 310—NEW DRUGS

Subpart A—General Provisions

Sec.
310.3 Definitions and interpretations.
310.4 Biologics; products subject to license control.
310.6 Applicability of ''new drug'' or safety or effectiveness findings in drug efficacy study implementation notices and notices of opportunity for hearing to identical, related, and similar drug products.

Subpart B—Specific Administrative Rulings and Decisions

310.100 New drug status opinions; statement of policy.
310.103 New drug substances intended for hypersensitivity testing.

Subpart C—New Drugs Exempted From Prescription-Dispensing Requirements

310.200 Prescription-exemption procedure.
310.201 Exemption for certain drugs limited by new drug applications to prescription sale.

Subpart D—Records and Reports

310.303 Continuation of long-term studies, records, and reports on certain drugs for which new drug applications have been approved.
310.305 Records and reports concerning adverse drug experiences on marketed prescription drugs for human use without approved new drug applications.

Subpart E—Requirements for Specific New Drugs or Devices

310.500 Digoxin products for oral use; conditions for marketing.
310.501 Patient package inserts for oral contraceptives.
310.502 Certain drugs accorded new drug status through rulemaking procedures.
310.503 Requirements regarding certain radioactive drugs.
310.504 Amphetamines (amphetamine, dextroamphetamine, and their salts and levamfetamine and its salts) for human use.
310.506 Use of vinyl chloride as an ingredient, including propellant, of aerosol drug products.
310.507 Aerosol drug products for human use containing 1,1,-1-trichloroethane.
310.508 Use of certain halogenated salicylanilides as an inactive ingredient in drug products.
310.509 Parenteral drug products in plastic containers.
310.510 Use of aerosol drug products containing zirconium.
310.513 Chloroform, use as an ingredient (active or inactive) in drug products.
310.515 Patient package inserts for estrogens.
310.516 Progestational drug products; labeling directed to the patient.
310.517 Labeling for oral hypoglycemic drugs of the sulfonylurea class.
310.518 Drug products containing iron or iron salts.
310.519 Drug products marketed as over-the-counter (OTC) daytime sedatives.
310.525 Sweet spirits of nitre drug products.
310.526 Camphorated oil drug products.
310.527 Drug products containing active ingredients offered over-the-counter (OTC) for external use as hair growers or for hair loss prevention.
310.528 Drug products containing active ingredients offered over-the-counter (OTC) for use as an aphrodisiac.
310.529 Drug products containing active ingredients offered over-the-counter (OTC) for oral use as insect repellents.
310.530 Topically applied hormone-containing drug products for over-the-counter (OTC) human use.
310.531 Drug products containing active ingredients offered over-the-counter (OTC) for the treatment of boils.
310.532 Drug products containing active ingredients offered over-the-counter (OTC) to relieve the symptoms of benign prostatic hypertrophy.
310.533 Drug products containing active ingredients offered over-the-counter (OTC) for human use as an anticholinergic in cough-cold drug products.
310.534 Drug products containing active ingredients offered over-the-counter (OTC) for human use as oral wound healing agents.
310.536 Drug products containing active ingredients offered over-the-counter (OTC) for use as a nailbiting or thumbsucking deterrent.
310.537 Drug products containing active ingredients offered over–the–counter (OTC) for oral administration for the treatment of fever blisters and cold sores.
310.538 Drug products containing active ingredients offered over-the-counter (OTC) for use for ingrown toenail relief.
310.540 Drug products containing active ingredients offered over-the-counter (OTC) for use as stomach acidifiers.
310.541 Over-the-counter (OTC) drug products containing active ingredients offered for use in the treatment of hypophosphatemia.
310.542 Over-the-counter (OTC) drug products containing active ingredients offered for use in the treatment of hyperphosphatemia.
310.543 Drug products containing active ingredients offered over-the-counter (OTC) for human use in exocrine pancreatic insufficiency.
310.544 Drug products containing active ingredients offered over-the-counter (OTC) for use as a smoking deterrent.
310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.
310.546 Drug products containing active ingredients offered over-the-counter (OTC) for the treatment and/or prevention of nocturnal leg muscle cramps.
310.547 Drug products containing quinine offered over-the-counter (OTC) for the treatment and/or prevention of malaria.

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b–360f, 360j, 361(a), 371, 374, 375, 379e; 42 U.S.C. 216, 241, 242(a), 262, 263b–263n.

Subpart A—General Provisions

§ 310.3 Definitions and interpretations.

As used in this part:

(a) The term *act* means the Federal Food, Drug, and Cosmetic Act, as amended (secs. 201–902, 52 Stat. 1040 et seq., as amended; 21 U.S.C. 321–392).

(b) *Department* means the Department of Health and Human Services.

(c) *Secretary* means the Secretary of Health and Human Services.

(d) *Commissioner* means the Commissioner of Food and Drugs.

(e) The term *person* includes individuals, partnerships, corporations, and associations.

(f) The definitions and interpretations of terms contained in section 201 of the act shall be applicable to such terms when used in the regulations in this part.

(g) *New drug substance* means any substance that when used in the manufacture, processing, or packing of a drug, causes that drug to be a new drug, but does not include intermediates used in the synthesis of such substance.

(h) The newness of a drug may arise by reason (among other reasons) of:

(1) The newness for drug use of any substance which composes such drug, in whole or in part, whether it be an active substance or a menstruum, excipient, carrier, coating, or other component.

(2) The newness for a drug use of a combination of two or more substances, none of which is a new drug.

(3) The newness for drug use of the proportion of a substance in a combination, even though such combination containing such substance in other proportion is not a new drug.

(4) The newness of use of such drug in diagnosing, curing, mitigating, treating, or preventing a disease, or to affect a structure or function of the body, even though such drug is not a new drug when used in another disease or to affect another structure or function of the body.

(5) The newness of a dosage, or method or duration of administration or application, or other condition of use prescribed, recommended, or suggested in the labeling of such drug, even though such drug when used in other dosage, or other method or duration of administration or application, or different condition, is not a new drug.

(i) [Reserved]

(j) The term *sponsor* means the person or agency who assumes responsibility for an investigation of a new drug, including responsibility for compliance with applicable provisions of the act and regulations. The ''sponsor'' may be an individual, partnership, corporation, or Government agency and may be a manufacturer, scientific institution, or an investigator regularly and lawfully engaged in the investigation of new drugs.

(k) The phrase *related drug(s)* includes other brands, potencies, dosage forms, salts, and esters of the same drug moiety, including articles prepared or manufactured by other manufacturers: and any other drug containing a component so related by chemical structure or known pharmacological properties that, in the opinion of experts qualified by scientific training and experience to evaluate the safety and effectiveness of drugs, it is prudent to assume or ascertain the liability of similar side effects and contraindications.

(l) *Special packaging* as defined in section 2(4) of the Poison Prevention Packaging Act of 1970 means packaging that is designed or constructed to be significantly difficult for children under 5 years of age to open or obtain a toxic or harmful amount of the substance contained therein within a reasonable time and not difficult for normal adults to use properly, but does not mean packaging which all such children cannot open or obtain a toxic or harmful amount within a reasonable time.

(m) [Reserved]

(n) The term *radioactive drug* means any substance defined as a drug in section 201(g)(1) of the Federal Food, Drug, and Cosmetic Act which exhibits spontaneous disintegration of unstable nuclei with the emission of nuclear particles or photons and includes any nonradioactive reagent kit or nuclide generator which is intended to be used in the preparation of any such substance but does not include drugs such as carbon-containing compounds or potassium-containing salts which contain trace quantities of naturally occurring radionuclides. The term ''radioactive drug'' includes a ''radioactive biological product'' as defined in § 600.3(ee) of this chapter.

[39 FR 11680, Mar. 29, 1974, as amended at 39 FR 20484, June 11, 1974; 40 FR 31307, July 25, 1975; 46 FR 8952, Jan. 27, 1981; 50 FR 7492, Feb. 22, 1985]

§ 310.4 Biologics; products subject to license control.

(a) Except for radioactive biological products intended for human use, a new drug shall not be deemed to be subject to section 505 of the act if it is a drug licensed under the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) or under the animal virus, serum, and toxin law of March 4, 1913 (37 Stat. 832 (21 U.S.C. 151 *et seq.*)).

(b) A radioactive biological product (as defined in § 600.3(ee) of this chapter) intended for human use is subject to section 505 of the act. Any license for such a radioactive biological product which is issued under the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) and which has not been revoked or suspended as of August 25, 1975 shall constitute an approved new drug application in effect under the same terms and conditions as set forth in such license and such portions of the establishment license relating to such product, which include data and information required under part 314 of this chapter for a new drug application. Any such radioactive biological product for which licensure under the Public Health Service Act is pending on August 25, 1975 shall, upon determination that it is acceptable for licensure, be approved as a new drug application in lieu of issuance of a biological product license.

[40 FR 31312, July 25, 1975]

§ 310.6 Applicability of "new drug" or safety or effectiveness findings in drug efficacy study implementation notices and notices of opportunity for hearing to identical, related, and similar drug products.

(a) The Food and Drug Administration's conclusions on the effectiveness of drugs are currently being published in the FEDERAL REGISTER as Drug Efficacy Study Implementation (DESI) Notices and as Notices of Opportunity for Hearing. The specific products listed in these notices include only those that were introduced into the market through the new drug procedures from 1938–62 and were submitted for review by the National Academy of Sciences-National Research Council (NAS–NRC), Drug Efficacy Study Group. Many products which are identical to, related to, or similar to the products listed in these notices have been marketed under different names or by different firms during this same period or since 1962 without going through the new drug procedures or the Academy review. Even though these products are not listed in the notices, they are covered by the new drug applications reviewed and thus are subject to these notices. All persons with an interest in a product that is identical, related, or similar to a drug listed in a drug efficacy notice or a notice of opportunity for a hearing will be given the same opportunity as the applicant to submit data and information, to request a hearing, and to participate in any hearing. It is not feasible for the Food and Drug Administration to list all products which are covered by an NDA and thus subject to each notice. However, it is essential that the findings and conclusions that a drug product is a ''new drug'' or that there is a lack of evidence to show that a drug product is safe or effective be applied to all identical, related, and similar drug products to which they are reasonably applicable. Any product not in compliance with an applicable drug efficacy notice is in violation of section 505 (new drugs) and/or section 502 (misbranding) of the act.

(b)(1) An identical, related, or similar drug includes other brands, potencies, dosage forms, salts, and esters of the same drug moiety as well as of any drug moiety related in chemical structure or known pharmacological properties.

(2) Where experts qualified by scientific training and experience to evaluate the safety and effectiveness of drugs would conclude that the findings and conclusions, stated in a drug efficacy notice or notice of opportunity for hearing, that a drug product is a ''new drug'' or that there is a lack of evidence to show that a drug product is safe or effective are applicable to an identical, related, or similar drug product, such product is affected by the notice. A combination drug product containing a drug that is identical, related, or similar to a drug named in a notice may also be subject to the findings and conclusions in a notice that a drug

product is a ''new drug'' or that there is a lack of evidence to show that a drug product is safe or effective.

(3) Any person may request an opinion on the applicability of such a notice to a specific product by writing to the Food and Drug Administration at the address shown in paragraph (e) of this section.

(c) Manufacturers and distributors of drugs should review their products as drug efficacy notices are published and assure that identical, related, or similar products comply with all applicable provisions of the notices.

(d) The published notices and summary lists of the conclusions are of particular interest to drug purchasing agents. These agents should take particular care to assure that the same purchasing policy applies to drug products that are identical, related, or similar to those named in the drug efficacy notices. The Food and Drug Administration applies the same regulatory policy to all such products. In many instances a determination can readily be made as to the applicability of a drug efficacy notice by an individual who is knowledgeable about drugs and their indications for use. Where the relationships are more subtle and not readily recognized, the purchasing agent may request an opinion by writing to the Food and Drug Administration at the address shown in paragraph (e) of this section.

(e) Interested parties may submit to the Food and Drug Administration, Center for Drug Evaluation and Research, Office of Compliance, HFD–300, 5600 Fishers Lane, Rockville, MD 20857, the names of drug products, and of their manufacturers or distributors, that should be the subject of the same purchasing and regulatory policies as those reviewed by the Drug Efficacy Study Group. Appropriate action, including referral to purchasing officials of various government agencies, will be taken.

(f) This regulation does not apply to OTC drugs identical, similar, or related to a drug in the Drug Efficacy Study unless there has been or is notification in the FEDERAL REGISTER that a drug will not be subject to an OTC panel review pursuant to §§ 330.10, 330.11, and 330.5 of this chapter.

[39 FR 11680, Mar. 29, 1974, as amended at 48 FR 2755, Jan. 21, 1983; 50 FR 8996, Mar. 6, 1985; 55 FR 11578, Mar. 29, 1990]

Subpart B—Specific Administrative Rulings and Decisions

§ 310.100 New drug status opinions; statement of policy.

(a) Over the years since 1938 the Food and Drug Administration has given informal advice to inquirers as to the new drug status of preparations. These drugs have sometimes been identified only by general statements of composition. Generally, such informal opinions were incorporated in letters that did not explicitly relate all of the necessary conditions and qualifications such as the quantitative formula for the drug and the conditions under which it was prescribed, recommended, or suggested. This has contributed to misunderstanding and misinterpretation of such opinions.

(b) These informal opinions that an article is ''not a new drug'' or ''no longer a new drug'' require reexamination under the Kefauver-Harris Act (Public Law 87–781; 76 Stat. 788–89). In particular, when approval of a new drug application is withdrawn under provisions of section 505(e) of the Federal Food, Drug, and Cosmetic Act, a drug generally recognized as safe may become a ''new drug'' within the meaning of section 201(p) of said act as amended by the Kefauver-Harris Act on October 10, 1962. This is of special importance by reason of proposed actions to withdraw approval of new drug applications for lack of substantial evidence of effectiveness as a result of reports of the National Academy of Sciences—National Research Council on its review of drug effectiveness; for example, see the notice published in the FEDERAL REGISTER of January 23, 1968 (33 FR 818), regarding rutin, quercetin, et al.

(c) Any marketed drug is a ''new drug'' if any labeling change made after October 9, 1962, recommends or suggests new conditions of use under which the drug is not generally recognized as safe and effective by qualified experts. Undisclosed or unreported side

effects as well as the emergence of new knowledge presenting questions with respect to the safety or effectiveness of a drug may result in its becoming a ''new drug'' even though it was previously considered ''not a new drug.'' Any previously given informal advice that an article is ''not a new drug'' does not apply to such an article if it has been changed in formulation, manufacture control, or labeling in a way that may significantly affect the safety of the drug.

(d) For these reasons, all opinions previously given by the Food and Drug Administration to the effect that an article is ''not a new drug'' or is ''no longer a new drug'' are hereby revoked. This does not mean that all articles that were the subjects of such prior opinions will be regarded as new drugs. The prior opinions will be replaced by opinions of the Food and Drug Administration that are qualified and current on when an article is ''not a new drug,'' as set forth in this subchapter.

[39 FR 11680, Mar. 29, 1974]

§ 310.103 New drug substances intended for hypersensitivity testing.

(a) The Food and Drug Administration is aware of the need in the practice of medicine for the ingredients of a new drug to be available for tests of hypersensitivity to such ingredients and therefore will not object to the shipment of a new drug substance, as defined in § 310.3(g), for such purpose if all of the following conditions are met:

(1) The shipment is made as a result of a specific request made to the manufacturer or distributor by a practitioner licensed by law to administer such drugs, and the use of such drugs for patch testing is not promoted by the manufacturer or distributor.

(2) The new drug substance requested is an ingredient in a marketed new drug and is not one that is an ingredient solely in a new drug that is legally available only under the investigational drug provisions of this part.

(3) The label bears the following prominently placed statements in lieu of adequate directions for use and in addition to complying with the other labeling provisions of the act:

(i) ''Caution: Federal law prohibits dispensing without a prescription''; and

(ii) ''For use only in patch testing''.

(4) The quantity shipped is limited to an amount reasonable for the purpose of patch testing in the normal course of the practice of medicine and is used solely for such patch testing.

(5) The new drug substance is manufactured by the same procedures and meets the same specifications as the component used in the finished dosage form.

(6) The manufacturer or distributor maintains records of all shipments for this purpose for a period of 2 years after shipment and will make them available to the Food and Drug Administration on request.

(b) When the requested new drug substance is intended for investigational use in humans or the substance is legally available only under the investigational drug provisions of part 312 of this chapter, the submission of an ''Investigational New Drug Application'' (IND) is required. The Food and Drug Administration will offer assistance to any practitioner wishing to submit an Investigational New Drug Application.

(c) This section does not apply to drugs or their components that are subject to the licensing requirements of the Public Health Service Act of 1944, as amended. (See subchapter F—Biologics, of this chapter.)

[39 FR 11680, Mar. 29, 1974, as amended at 55 FR 11578, Mar. 29, 1990]

Subpart C—New Drugs Exempted From Prescription-Dispensing Requirements

§ 310.200 Prescription-exemption procedure.

(a) *Duration of prescription requirement.* Any drug limited to prescription use under section 503(b)(1)(C) of the act remains so limited until it is exempted as provided in paragraph (b) or (e) of this section.

(b) *Prescription-exemption procedure for drugs limited by a new drug application.* Any drug limited to prescription use under section 503(b)(1)(C) of the act shall be exempted from prescription-dispensing requirements when the Commissioner finds such requirements are not necessary for the protection of the public health by reason of the

drug's toxicity or other potentiality for harmful effect, or the method of its use, or the collateral measures necessary to its use, and he finds that the drug is safe and effective for use in self-medication as directed in proposed labeling. A proposal to exempt a drug from the prescription-dispensing requirements of section 503(b)(1)(C) of the act may be initiated by the Commissioner or by any interested person. Any interested person may file a petition seeking such exemption, which petition may be pursuant to part 10 of this chapter, or in the form of a supplement to an approved new drug application.

(c) *New drug status of drugs exempted from the prescription requirement.* A drug exempted from the prescription requirement under the provisions of paragraph (b) of this section is a ''new drug'' within the meaning of section 201(p) of the act until it has been used to a material extent and for a material time under such conditions except as provided in paragraph (e) of this section.

(d) *Prescription legend not allowed on exempted drugs.* The use of the prescription caution statement quoted in section 503(b) (4) of the act, in the labeling of a drug exempted under the provisions of this section, constitutes misbranding. Any other statement or suggestion in the labeling of a drug exempted under this section, that such drug is limited to prescription use, may constitute misbranding.

(e) *Prescription-exemption procedure of OTC drug review.* A drug limited to prescription use under section 503(b)(1)(C) of the act may also be exempted from prescription-dispensing requirements by the procedure set forth in § 330.13 of this chapter.

[39 FR 11680, Mar. 29, 1974, as amended at 41 FR 32582, Aug. 4, 1976; 42 FR 4714, Jan. 25, 1977; 42 FR 15674, Mar. 22, 1977]

§ 310.201 Exemption for certain drugs limited by new-drug applications to prescription sale.

(a) The prescription-dispensing requirements of section 503(b)(1)(C) of the Federal Food, Drug, and Cosmetic Act are not necessary for the protection of the public health with respect to the following drugs subject to new drug applications:

(1) *N*-Acetyl-*p*-aminophenol (acetaminophen, *p*-hydroxy-acetanilid) preparations meeting all the following conditions:

(i) The *N*-acetyl-*p*-aminophenol is prepared, with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The *N*-acetyl-*p*-aminophenol and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505 (b) of the act is approved for it.

(iv) The preparation contains not more than 0.325 gram (5 grains) of *N*-acetyl-*p*-aminophenol per dosage unit, or if it is in liquid form not more than 100 milligrams of *N*-acetyl-*p*-aminophenol per milliliter.

(v) The preparation is labeled with adequate directions for use in minor conditions as a simple analgesic.

(vi) The dosages of *N*-acetyl-*p*-aminophenol recommended or suggested in the labeling do not exceed: For adults, 0.65 gram (10 grains) per dose or 2.6 grams (40 grains) per 24-hour period: for children 6 to 12 years of age, one-half of the maximum adult dose or dosage; for children 3 to 6 years of age, one-fifth of the maximum adult dose or dosage.

(vii) The labeling bears, in juxtaposition with the dosage recommendations, a clear warning statement against administration of the drug to children under 3 years of age and against use of the drug for more than 10 days, unless such uses are directed by a physician.

(viii) If the article is offered for use in arthritis or rheumatism, the labeling prominently bears a statement that the beneficial effects claimed are limited to the temporary relief of minor aches and pains of arthritis and rheumatism and, in juxtaposition with directions for use in such conditions, a conspicuous warning statement, such as ''Caution: If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age, consult a physician immediately''.

(2) Sodium gentisate (sodium-2, 5-dihydroxybenzoate) preparations meeting all the following conditions:

(i) The sodium gentisate is prepared, with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The sodium gentisate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 0.5 gram (7.7 grains) of anhydrous sodium gentisate per dosage unit.

(v) The preparation is labeled with adequate directions for use in minor conditions as a simple analgesic.

(vi) The dosages of sodium gentisate recommended or suggested in the labeling do not exceed: For adults, 0.5 gram (7.7 grains) per dose of 2.0 grams (31 grains) per 24-hour period; for children 6 to 12 years of age, one-half of the maximum adult dose or dosage.

(vii) The labeling bears, in juxtaposition with the dosage recommendations, a clear warning statement against administration of the drug to children under 6 years of age and against use of the drug for a prolonged period, except as such uses may be directed by a physician.

(3) Isoamylhydrocupreine and zolamine hydrochloride (N, N-dimethyl-N′-2-thiazolyl-N′-*p*-methoxybenzyl-ethylenediamine hydrochloride) preparations meeting all the following conditions:

(i) The isoamylhydrocupreine and zolamine hydrochloride are prepared in dosage form suitable for self-medication as rectal suppositories or as an ointment and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The isoamylhydrocupreine, zolamine hydrochloride, and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 0.25 percent of isoamylhydrocupreine and 1.0 percent of zolamine hydrochloride.

(v) If the preparation is in suppository form, it contains not more than 5.0 milligrams of isoamylhydrocupreine and not more than 20.0 milligrams of zolamine hydrochloride per suppository.

(vi) The preparation is labeled with adequate directions for use in the temporary relief of local pain and itching associated with hemorrhoids.

(vii) The directions provide for the use of not more than two suppositories or two applications of ointment in a 24-hour period.

(viii) The labeling bears, in juxtaposition with the dosage recommendations, a clear warning statement against use of the preparation in case of rectal bleeding, as this may indicate serious disease.

(4) Phenyltoloxamine dihydrogen citrate (*N*,*N*-dimethyl-(*a*-phenyl-*O*-toloxy) ethylamine dihydrogen citrate), preparations meeting all the following conditions:

(i) The phenyltoloxamine dihydrogen citrate is prepared, with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The phenyltoloxamine dihydrogen citrate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 88 milligrams of phenyltoloxamine dihydrogen citrate (equivalent to 50 milligrams of phenyltoloxamine) per dosage unit.

(v) The preparation is labeled with adequate directions for use in the temporary relief of the symptoms of hay fever and/or the symptoms of other minor conditions in which it is indicated.

(vi) The dosages recommended or suggested in the labeling do not exceed: For adults, 88 milligrams of phenyltoloxamine dihydrogen citrate (equivalent to 50 milligrams of phenyltoloxamine) per dose or 264 milligrams of phenyltoloxamine dihydrogen citrate (equivalent to 150 milligrams of phenyltoloxamine) per 24-hour period; for children 6 to 12 years of age, one-half of the maximum adult dose or dosage.

(vii) The labeling bears, in juxtaposition with the dosage recommendations:

(*a*) Clear warning statements against administration of the drug to children under 6 years of age, except as directed by a physician, and against driving a car or operating machinery while using the drug, since it may cause drowsiness.

(*b*) If the article is offered for temporary relief of the symptoms of colds, a statement that continued administration for such use should not exceed 3 days, except as directed by a physician.

(5)–(7) [Reserved]

(8) Dicyclomine hydrochloride (1-cyclohexylhexahydrobenzoic acid. β-diethylaminoethyl ester hydrochloride; diethylaminocarbethoxy-bicyclohexyl hydrochloride) preparations meeting all the following conditions:

(i) The dicyclomine hydrochloride is prepared with suitable antacid and other components, in tablet or other dosage form for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The dicyclomine hydrochloride and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 5 milligrams of dicyclomine hydrochloride per dosage unit, or if it is in liquid form not more than 0.5 milligram of dicyclomine hydrochloride per milliliter.

(v) The preparation is labeled with adequate directions for use only by adults and children over 12 years of age, in the temporary relief of gastric hyperacidity.

(vi) The dosages recommended or suggested in the directions for use do not exceed 10 milligrams of dicyclomine hydrochloride per dose or 30 milligrams in a 24-hour period.

(vii) The labeling bears, in juxtaposition with the dosage recommendations, clear warning statements against:

(*a*) Exceeding the recommended dosage.

(*b*) Prolonged use, except as directed by a physician, since persistent or recurring symptoms may indicate a serious disease requiring medical attention.

(*c*) Administration to children under 12 years of age except as directed by a physician.

(9)–(10) [Reserved]

(11) Hexadenol (a mixture of tetracosanes and their oxidation products) preparations meeting all the following conditions:

(i) The hexadenol is prepared and packaged, with or without other drugs, solvents, and propellants, in a form suitable for self-medication by external application to the skin as a spray, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The hexadenol and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 5 percent by weight of hexadenol.

(v) The preparation is labeled with adequate directions for use by external application in the treatment of minor burns and minor skin irritations.

(vi) The labeling bears, in juxtaposition with the directions for use, clear warning statements against:

(*a*) Use on serious burns or skin conditions or prolonged use, except as directed by a physician.

(*b*) Spraying the preparation in the vicinity of eyes, mouth, nose, or ears.

(12) Sulfur dioxide preparations meeting all the following conditions:

(i) The sulfur dioxide is prepared with or without other drugs, in an aqueous

solution packaged in a hermetic container suitable for use in self-medication by external application to the skin, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The sulfur dioxide and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 5 grams of sulfur dioxide per 100 milliliters of solution.

(v) The preparation is labeled with adequate directions for use by external application to the smooth skin in the prevention or treatment of minor conditions in which it is indicated.

(vi) The directions for use recommend or suggest not more than two applications a day for not more than 1 week, except as directed by a physician.

(13)–(15) [Reserved]

(16) Tuaminoheptane sulfate (2-aminoheptane sulfate) preparations meeting all the following conditions:

(i) The tuaminoheptane sulfate is prepared, with or without other drugs, in an aqueous vehicle suitable for administration in self-medication as nose drops, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The preparation is packaged with a style of container or assembly suited to self-medication by the recommended route of administration, and delivering not more than 0.1 milliliter of the preparation per drop.

(iii) The tuaminoheptane sulfate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iv) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(v) The tuaminoheptane sulfate content of the preparation does not exceed 10 milligrams per milliliter.

(vi) The preparation is labeled with adequate directions for use in the temporary relief of nasal congestion.

(vii) The dosages recommended or suggested in the directions for use do not exceed the equivalent: For adults, 5 drops of a 1 percent solution per nostril per dose, and 5 doses in a 24-hour period; for children 1 to 6 years of age, 3 drops of a 1 percent solution per nostril per dose, and 5 doses in a 24-hour period; for infants under 1 year of age, 2 drops of a 1 percent solution per nostril per dose, and 5 doses in a 24-hour period.

(viii) The labeling bears, in juxtaposition with the dosage recommendations:

(*a*) Clear warning statements against use of more than 5 doses daily, and against use longer than 4 days unless directed by a physician.

(*b*) A clear warning statement to the effect that frequent use may cause nervousness or sleeplessness, and that individuals with high blood pressure, heart disease, diabetes, or thyroid disease should not use the preparation unless directed by a physician.

(17) [Reserved]

(18) Vibesate (a mixture of copolymers of hydroxy-vinyl chlorideacetate, sebacic acid, and modified maleic rosin ester) preparations meeting all the following conditions.

(i) The vibesate is prepared and packaged, with or without other drugs, solvents, and propellants, in a form suitable for self-medication by external application to the skin as a spray, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The vibesate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 13 percent by weight of vibesate.

(v) The preparation is labeled with adequate directions for use by external application as a dressing for minor burns, minor cuts, or other minor skin irritations.

(vi) The labeling bears in juxtaposition with the directions for use clear warning statements against:

(*a*) Use on serious burns and on infected, deep, and puncture wounds unless directed by a physician.

(*b*) Spraying the preparation near the eyes or other mucous membranes.

(*c*) Inhaling the preparation.

(*d*) Use near open flames.

(*e*) Puncturing the container or throwing the container into fire.

(19) Pramoxine hydrochloride (4-*N*-butoxyphenyl γ-morpholinopropyl ether hydrochloride) preparations meeting all the following conditions:

(i) The pramoxine hydrochloride is prepared, with or without other drugs, in a dosage form suitable for use in self-medication by external application to the skin, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The pramoxine hydrochloride and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 1.0 percent of pramoxine hydrochloride.

(v) The preparation is labeled with adequate directions for use by external application to the skin for the temporary relief of pain or itching due to minor burns and sunburn, nonpoisonous insect bites, and minor skin irritations.

(vi) The directions for use recommend or suggest not more than four applications of the preparation per day, unless directed by a physician.

(vii) The labeling bears, in juxtaposition with the directions for use, clear warning statements against:

(*a*) Prolonged use.

(*b*) Application to large areas of the body.

(*c*) Continued use if redness, irritation, swelling, or pain persists or increases, unless directed by a physician.

(*d*) Use in the eyes or nose.

(20) Carbetapentane citrate (2-(2-diethylaminoethoxy)-ethyl-1-phenylcyclopentyl-1-carboxylate citrate) preparations meeting all the following conditions:

(i) The carbetanentane citrate is prepared, with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The carbetapentane citrate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, and application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 25 milligrams of carbetapentane citrate per dosage unit; or if it is in liquid form, not more than 1.5 milligrams of carbetapentane citrate per milliliter.

(v) The preparation is labeled with adequate directions for use in the temporary relief of cough due to minor conditions in which it is indicated.

(vi) The dosages recommended or suggested in the labeling do not exceed: For adults, 30 milligrams of carbetapentane citrate per dose or 120 milligrams of carbetapentane citrate per 24-hour period; for children 4 to 12 years of age, 7.5 milligrams per dose or 30 milligrams per 24-hour period; for children 2 to 4 years of age, 4.0 milligrams per dose or 16.0 milligrams per 24-hour period.

(vii) The label bears a conspicuous warning to keep the drug out of the reach of children, and the labeling bears, in juxtaposition with the dosage recommendations:

(*a*) A clear warning statement against administration of the drug to children under 2 years of age, unless directed by a physician.

(*b*) Clear warning statements against use of the drug in the presence of high fever or if cough persists, since persistent cough as well as high fever may indicate the presence of a serious condition.

(21) Pamabrom (2-amino-2-methylpropanol-1-8-bromotheophyllinate) preparations meeting all the following conditions:

(i) The pamabrom is prepared with appropriate amounts of a suitable analgesic and with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The pamabrom and all other components of the preparation meet their

professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 50 milligrams of pamabrom per dosage unit.

(v) The preparation is labeled with adequate directions for use in the temporary relief of the minor pains and discomforts that may occur a few days before and during the menstrual period.

(vi) The dosages recommended or suggested in the labeling do not exceed 50 milligrams of pamabrom per dose or 200 milligrams per 24-hour period.

(22) Diphemanil methylsulfate (4-diphenylmethylene-1,1-dimethyl-piperidinium methylsulfate) preparations meeting all the following conditions:

(i) The diphemanil methylsulfate is prepared, with or without other drugs, in a dosage form suitable for use in self-medication by external application to the skin, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The diphemanil methylsulfate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 2.0 percent of diphemanil methylsulfate.

(v) The preparation is labeled with adequate directions for use by external application to the skin for the relief of symptoms of mild poison ivy, oak, and sumac and other minor irritations and itching of the skin.

(vi) The directions for use recommend or suggest not more than four applications of the preparation per day, unless directed by a physician.

(vii) The labeling bears, in juxtaposition with the directions for use, a clear warning statement, such as: ''Caution: If redness, irritation, swelling, or pain persists or increases, discontinue use and consult physician.''

(23) Dyclonine hydrochloride (4-butoxy-3-piperidinopropiophenone hydrochloride; 4-*n*-butoxy-β-piperidonopropiophenone hydrochloride) preparations meeting all the following conditions:

(i) The dyclonine hydrochloride is prepared, with or without other drugs, in a dosage form suitable for use as a cream or ointment in self-medication by external application to the skin, or rectally, and contains no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The dyclonine hydrochloride and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 1.0 percent of dyclonine hydrochloride.

(v) The preparation is labeled with adequate directions for use:

(*a*) By external application to the skin for the temporary relief of pain and itching in sunburn, nonpoisonous insect bites, minor burns, cuts, abrasions, and other minor skin irritations.

(*b*) [Reserved]

(*c*) In the prevention or treatment of other minor conditions in which it is indicated.

(vi) The labeling bears, in juxtaposition with the directions for use, clear warning statements against:

(*a*) Continued use if redness, irritation, swelling, or pain persists or increases, unless directed by a physician.

(*b*) Use in case of rectal bleeding, as this may indicate serious disease.

(*c*) Use in the eyes.

(*d*) Prolonged use.

(*e*) Application to large areas of the body.

(*f*) Use for deep or puncture wounds or serious burns.

(24) Chlorothen citrate (chloromethapyrilene citrate; *N,N*-dimethyl-*N′*-(2-pyridyl)-*N ′*-(5-chloro-2-thenyl) ethylenediamine citrate) preparations meeting all the following conditions:

(i) The chlorothen citrate is prepared, with or without other drugs, in tablet or other dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The chlorothen citrate and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 25 milligrams of chlorothen citrate per dosage unit.

(v) The preparation is labeled with adequate directions for use in the temporary relief of the symptoms of hay fever and/or the symptoms of other minor conditions in which it is indicated.

(vi) The dosages recommended or suggested in the labeling do not exceed: For adults, 25 milligrams of chlorothen citrate per dose or 150 milligrams of chlorothen citrate per 24-hour period; for children 6 to 12 years of age, one-half of the maximum adult dose or dosage.

(vii) The labeling bears, in juxtaposition with the dosage recommendations:

(*a*) Clear warning statements against administration of the drug to children under 6 years of age or exceeding the recommended dosage, unless directed by a physician, and against driving a car or operating machinery while using the drug, since it may cause drowsiness.

(*b*) If the article is offered for the temporary relief of symptoms of colds, a statement that continued administration for such use should not exceed 3 days, unless directed by a physician.

(25) [Reserved]

(26) Methoxyphenamine hydrochloride (β-(*o*-methoxyphenyl)-isopropyl-methylamine hydrochloride; 1-(*o*-methoxyphenyl)-2-methylamino-propane hydrochloride) preparations meeting all the following conditions:

(i) The methoxyphenamine hydrochloride is prepared with appropriate amounts of a suitable antitussive, with or without other drugs, in a dosage form suitable for oral use in self-medication, and containing no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The methoxyphenamine hydrochloride and all other components of the preparation meet their professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 3.5 milligrams of methoxyphenamine hydrochloride per milliliter.

(v) The preparation is labeled with adequate directions for use in the temporary relief of cough due to minor conditions in which it is indicated.

(vi) The dosages recommended or suggested in the labeling do not exceed: For adults, 35 milligrams of methoxyphenamine hydrochloride per dose or 140 milligrams of methoxyphenamine hydrochloride per 24-hour period; for children 6 to 12 years of age, one-half of the maximum adult dose or dosage.

(vii) The label bears a conspicuous warning to keep the drug out of the reach of children, and the labeling bears, in juxtaposition with the dosage recommendations:

(*a*) A clear warning statement against administration of the drug to children under 6 years of age, unless directed by a physician.

(*b*) A clear warning statement to the effect that frequent or prolonged use may cause nervousness, restlessness, or drowsiness, and that individuals with high blood pressure, heart disease, diabetes, or thyroid disease should not use the preparation unless directed by a physician.

(*c*) A clear warning statement against use of the drug in the presence of high fever or if cough persists, since persistent cough as well as high fever may indicate the presence of a serious condition.

(27) Biphenamine hydrochloride (β-diethylaminoethyl-3-phenyl-2-hydroxybenzoate hydrochloride) preparations meeting all the following conditions:

(i) The biphenamine hydrochloride is prepared in a form suitable for use as a shampoo and contains no drug limited to prescription sale under the provisions of section 503(b)(1) of the act.

(ii) The biphenamine hydrochloride meets its professed standards of identity, strength, quality, and purity.

(iii) If the preparation is a new drug, an application pursuant to section 505(b) of the act is approved for it.

(iv) The preparation contains not more than 1 percent of biphenamine hydrochloride.

(v) The preparation is labeled with adequate directions for use for the temporary relief of itching and scaling due to dandruff.

(vi) The label bears a conspicuous warning to keep the drug out of the reach of children.

(28) Tyloxapol (an alkylarylpolyether alcohol) and benzalkonium chloride ophthalmic preparations meeting all the following conditions:

(i) The tyloxapol and benzalkonium chloride are prepared, with other appropriate ingredients which are not drugs limited to prescription sale under the provisions of section 503(b)(1) of the act, as a sterile, isotonic aqueous solution suitable for use in self-medication on eye prostheses.

(ii) The preparation is so packaged as to volume and type of container as to afford adequate protection and be suitable for self-medication with a minimum risk of contamination of the solution during use. Any dispensing unit is sterile and so packaged as to maintain sterility until the package is opened.

(iii) The tyloxapol, benzalkonium chloride, and other ingredients used to prepare the isotonic aqueous solution meet their professed standards of identity, strength, quality, and purity.

(iv) An application pursuant to section 505(b) of the act is approved for the drug.

(v) The preparation contains 0.25 percent of tyloxapol and 0.02 percent of benzalkonium chloride.

(vi) The label bears a conspicuous warning to keep the drug out of the reach of children and the labeling bears, in juxtaposition with the dosage recommendations, a clear warning that if irritation occurs, persists, or increases, use of the drug should be discontinued and a physician consulted. The labeling includes a statement that the dropper or other dispensing tip should not touch any surface, since this may contaminate the solution.

(29) [Reserved]

(b) [Reserved]

[39 FR 11680, Mar. 29, 1974, as amended at 42 FR 36994, July 19, 1977; 52 FR 15892, Apr. 30, 1987; 52 FR 30055, Aug. 12, 1987; 55 FR 31779, Aug. 3, 1990; 57 FR 58374, Dec. 9, 1992; 58 FR 49898, Sept. 23, 1993; 59 FR 4218, Jan. 28, 1994; 60 FR 52507, Oct. 6, 1995]

Subpart D—Records and Reports

§ 310.303 Continuation of long-term studies, records, and reports on certain drugs for which new drug applications have been approved.

(a) A new drug may not be approved for marketing unless it has been shown to be safe and effective for its intended use(s). After approval, the applicant is required to establish and maintain records and make reports related to clinical experience or other data or information necessary to make or facilitate a determination of whether there are or may be grounds under section 505(e) of the act for suspending or withdrawing approval of the application. Some drugs, because of the nature of the condition for which they are intended, must be used for long periods of time—even a lifetime. To acquire necessary data for determining the safety and effectiveness of long-term use of such drugs, extensive animal and clinical tests are required as a condition of approval. Nonetheless, the therapeutic or prophylactic usefulness of such drugs may make it inadvisable in the public interest to delay the availability of the drugs for widespread clinical use pending completion of such long-term studies. In such cases, the Food and Drug Administration may approve the new drug application on condition that the necessary long-term studies will be conducted and the results recorded and reported in an organized fashion. The procedures required by paragraph (b) of this section will be followed in order to list such a drug in § 310.304.

(b) A proposal to require additional or continued studies with a drug for which a new drug application has been approved may be made by the Commissioner on his own initiative or on the petition of any interested person, pursuant to part 10 of this chapter. Prior

to issuance of such a proposal, the applicant will be provided an opportunity for a conference with representatives of the Food and Drug Administration. When appropriate, investigators or other individuals may be invited to participate in the conference. All requirements for special studies, records, and reports will be published in § 310.304.

[39 FR 11680, Mar. 29, 1974, as amended at 41 FR 4714, Jan. 25, 1976; 42 FR 15674, Mar. 22, 1977]

§ 310.305 Records and reports concerning adverse drug experiences on marketed prescription drugs for human use without approved new drug applications.

(a) *Scope.* FDA is requiring manufacturers, packers, and distributors of marketed prescription drug products that are not the subject of an approved new drug or abbreviated new drug application to establish and maintain records and make reports to FDA of all serious, unexpected adversedrug experiences associated with the use of their drug products. Any person subject to the reporting requirements of paragraph (c) of this section shall also develop written procedures for the surveillance, receipt, evaluation, and reporting of postmarketing adverse drug experiences to FDA.

(b) *Definitions.* The following definitions of terms apply to this section:–

Adverse drug experience. Any adverse event associated with the use of a drug in humans, whether or not considered drug related, including the following: An adverse event occurring in the course of the use of a drug product in professional practice; an adverse event occurring from drug overdose whether accidental or intentional; an adverse event occurring from drug abuse; an adverse event occurring from drug withdrawal; and any failure of expected pharmacological action.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient, in the view of the initial reporter, at *immediate* risk of death from the adverse drug experience as it occurred, i.e., it does not include an adverse drug experience that, had it occurred in a more severe form, might have caused death.

Serious adverse drug experience. Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience. Any adverse drug experience that is not listed in the current labeling for the drug product. This includes events that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differ from the event because of greater severity or specificity. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the labeling only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the labeling only listed cerebral vascular accidents. ''Unexpected,'' as used in this definition, refers to an adverse drug experience that has not been previously observed (i.e., included in the labeling) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(c) *Reporting requirements.* Each person identified in paragraph (c)(1)(i) of this section shall report to FDA adverse drug experience information as

described in this section and shall submit one copy of each report to the Division of Pharmacovigilance and Epidemiology (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(1) *Postmarketing 15-day ''Alert reports''.* (i) Any person whose name appears on the label of a marketed prescription drug product as its manufacturer, packer, or distributor shall report to FDA each adverse drug experience received or otherwise obtained that is both serious and unexpected as soon as possible, but in no case later than 15 calendar days of initial receipt of the information by the person whose name appears on the label. Each report shall be accompanied by a copy of the current labeling for the drug product.

(ii) A person identified in paragraph (c)(1)(i) of this section is not required to submit a 15-day ''Alert report'' for an adverse drug experience obtained from a postmarketing study (whether or not conducted under an investigational new drug application) unless the applicant concludes that there is a reasonable possibility that the drug caused the adverse experience.

(2) *Postmarketing 15-day ''Alert reports''—followup.* Each person identified in paragraph (c)(1)(i) of this section shall promptly investigate all serious, unexpected adverse drug experiences that are the subject of these postmarketing 15-day Alert reports and shall submit followup reports within 15 calendar days of receipt of new information or as requested by FDA. If additional information is not obtainable, records should be maintained of the unsuccessful steps taken to seek additional information. Postmarketing 15-day Alert reports and followups to them shall be submitted under separate cover.

(3) *Submission of reports.* To avoid unnecessary duplication in the submission of, and followup to, reports required in this section, a packer's or distributor's obligations may be met by submission of all reports of serious adverse drug experiences to the manufacturer of the drug product. If a packer or distributor elects to submit these adverse drug experience reports to the manufacturer rather than to FDA, it shall submit each report to the manufacturer within 5 calendar days of its receipt by the packer or distributor, and the manufacturer shall then comply with the requirements of this section even if its name does not appear on the label of the drug product. Under this circumstance, the packer or distributor shall maintain a record of this action which shall include:

(i) A copy of each adverse drug experience report;

(ii) The date the report was received by the packer or distributor;

(iii) The date the report was submitted to the manufacturer; and

(iv) The name and address of the manufacturer.

(4) Each report submitted to FDA under this section shall bear prominent identification as to its contents, i.e., ''15-day Alert report,'' or ''15-day Alert report-followup.''

(5) A person identified in paragraph (c)(1)(i) of this section is not required to resubmit to FDA adverse drug experience reports forwarded to that person by FDA; however, the person must submit all *followup* information on such reports to FDA.

(d) *Reporting form.* (1) Except as provided in paragraph (d)(3) of this section, each person identified in paragraph (c)(1)(i) of this section shall submit each report of a serious and unexpected adverse drug experience on an FDA Form 3500A (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form).

(2) Each completed FDA Form 3500A should pertain only to an individual patient.

(3) Instead of using Form FDA Form 3500A, a manufacturer, packer, or distributor may use a computer-generated FDA Form 3500A or other alternative format (e.g., a computer-generated tape or tabular listing) provided that:

(i) The content of the alternative format is equivalent in all elements of information to those specified in FDA Form 3500A, and

(ii) The format is agreed to in advance by MedWatch: The FDA Medical Products Reporting Program.

(4) Ten copies or fewer of FDA Form 3500A and/or a copy of the instructions

for completing the form may be obtained from the Division of Pharmacovigilance and Epidemiology (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. More than 10 copies of the form may be obtained by writing to the Consolidated Forms and Publications Distribution Center, Washington Commerce Center, 3222 Hubbard Rd., Landover, MD 20785.

(e) *Patient privacy.* Manufacturers, packers, and distributors should not include in reports under this section the names and addresses of individual patients; instead, the manufacturer, packer, and distributor should assign a unique code number to each report, preferably not more than eight characters in length. The manufacturer, packer, and distributor should include the name of the reporter from whom the information was received. Names of patients, individual reporters, health care professionals, hospitals, and geographical identifiers in adverse drug experience reports are not releasable to the public under FDA's public information regulations in part 20 of this chapter.

(f) *Recordkeeping.* (1) Each manufacturer, packer, and distributor shall maintain for a period of 10 years records of all adverse drug experiences required under this section to be reported, including raw data and any correspondence relating to the adverse drug experiences, and the records required to be maintained under paragraph (c)(4) of this section.

(2) Manufacturers and packers may retain the records required in paragraph (f)(1) of this section as part of its complaint files maintained under § 211.198 of this chapter.

(3) Manufacturers, packers, and distributors shall permit any authorized FDA employee, at all reasonable times, to have access to and copy and verify the records established and maintained under this section.

(g) *Disclaimer.* A report or information submitted by a manufacturer, packer, or distributor under this section (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the manufacturer, packer, or distributor, or by FDA, that the report or information constitutes an admission that the drug caused or contributed to an adverse effect. The manufacturer, packer, or distributor need not admit, and may deny, that the report or information submitted under this section constitutes an admission that the drug caused or contributed to an adverse effect.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0210)

[51 FR 24779, July 3, 1986, as amended at 52 FR 37936, Oct. 13, 1987; 55 FR 11578, Mar. 29, 1990; 57 FR 17980, Apr. 28, 1992; 62 FR 34167, June 25, 1997; 62 FR 52249, Oct. 7, 1997]

EFFECTIVE DATE NOTE: At 62 FR 52249, Oct. 7, 1997, § 310.305 was amended by adding a new sentence at the end of paragraph (a); by revising paragraphs (b), (c), (d)(1), (d)(3)(ii), and (d)(4); by removing in paragraph (d)(2), the introductory text of paragraph (d)(3), and paragraph (d)(3)(i) the words ''Form FDA–1639'' or ''FDA–1639'' and adding in their place ''FDA Form 3500A'', effective Apr. 6, 1998. For the convenience of the user, the superseded text is set forth as follows:

§ 310.305 Records and reports concerning adverse drug experiences on marketed prescription drugs for human use without approved new drug applications.

* * * * *

(b) *Definitions.* The following definitions of terms apply to this section:

(1) *FDA* means the Food and Drug Administration.

(2) *Adverse drug experience* means any adverse event associated with the use of a drug in humans, whether or not considered drug related, including the following: an adverse event occurring in the course of the use of a drug product in professional practice; an adverse event occurring from drug overdose, whether accidental or intentional; an adverse event occurring from drug abuse; an adverse event occurring from drug withdrawal; and any failure of expected pharmacological action.

(3) *Unexpected* means an adverse drug experience that is not listed in the current labeling for the drug product and includes an event that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differs from the event because of greater severity or specificity. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the labeling only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism

and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the labeling only listed cerebral vascular accidents.

(4) *Serious* means an adverse drug experience that is fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

(c) *Reporting requirements—15-day ''Alert reports.''* (1)(i) Any person whose name appears on the label of a marketed prescription drug product as its manufacturer, packer, or distributor shall report to FDA each adverse drug experience received or otherwise obtained that is both serious and unexpected as soon as possible but in any case within 15 working days of initial receipt of the information. Each report shall be accompanied by a copy of the current labeling for the drug product.

(ii) A person identified in paragraph (c)(1)(i) of this section is not required to submit a 15-day ''Alert report'' for an adverse drug experience obtained from a postmarketing study (whether or not conducted under an investigational new drug application) unless the applicant concludes that there is a reasonable possibility that the drug caused the adverse experience.

(2) Each person identified in paragraph (c)(1) of this section shall submit one copy of each report to the Division of Epidemiology and Surveillance (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(3) Each person identified in paragraph (c)(1) of this section shall promptly investigate all serious, unexpected adverse drug experiences that are the subject of these 15-day Alert reports and shall submit followup reports within 15 working days of receipt of new information or as requested by FDA. If additional information is not obtainable, a followup report may be required that describes briefly the steps taken to seek additional information and the reasons why it could not be obtained.

(4) To avoid unnecessary duplication in the submission of, and followup to, reports required in this section, a packer's or distributor's obligations may be met by submission of all reports of serious adverse drug experiences to the manufacturer of the drug product. If a packer or distributor elects to submit these adverse drug experience reports to the manufacturer rather than to FDA, it shall submit each report to the manufacturer within 3 working days of its receipt by the packer or distributor, and the manufacturer shall then comply with the requirements of this section even if its name does not appear on the label of the drug product. Under this circumstance, the packer or distributor shall maintain a record of this action which shall include:

(i) A copy of each drug experience report.

(ii) Date the report was received by the packer or distributor.

(iii) Date the report was submitted to the manufacturer.

(iv) Name and address of the manufacturer.

(5) Each report submitted to FDA under this section shall bear prominent identification as to its contents, i.e., ''15-day Alert report'' or ''15-day Alert report—followup.''

(d) *Reporting form.* (1) Except as provided in paragraph (d)(3) of this section, each person identified in paragraph (c)(1) of this section shall submit each report of a serious and unexpected adverse drug experience on a Form FDA-1639 (Adverse Reaction Report).

* * * * *

(3)* * *

(i)* * *

(ii) The format is agreed to in advance by the Division of Epidemiology and Surveillance (HFD–730).

(4) Single copies of Form FDA–1639 may be obtained from the Division of Epidemiology and Surveillance (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Supplies of Form FDA–1639 may be obtained from the PHS Forms and Publications Distribution Center, 12100 Parklawn Dr., Rockville, MD 20857.

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Subpart E—Requirements for Specific New Drugs or Devices

§ 310.500 Digoxin products for oral use; conditions for marketing.

(a) Studies have shown evidence of clinically significant differences in bioavailability in different batches of certain marketed digoxin products for oral use from single manufacturers as well as in batches of these products produced by different manufacturers. These differences were observed despite the fact that the products met compendial specifications. Other studies have shown that there is a sufficient correlation between bioavailability in vivo and the dissolution rate of digoxin tablets in vitro to make the dissolution test an important addition to the compendial standards. Because of the potential for serious risk to cardiac patients using digoxin products which may vary in bioavailability, the Commissioner of Food and Drugs has determined that immediate action must be taken to assure the uniformity

of all digoxin products for oral use. The Commissioner is of the opinion that digoxin products for oral use are new drugs within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act for which approved new drug applications are required. The Commissioner has determined that, because of questions raised regarding the bioavailability of digoxin products for oral use, there is sufficient evidence to invoke the authority under section 505(j) of the act to fully investigate this question and to facilitate a determination of whether there is a ground for withdrawal of approval of the drug product under section 505(e) of the act. Marketing of these products may be continued only under the following conditions:

(1) Digoxin products for oral use, other than tablets: Any person marketing digoxin products for oral use, other than tablets, shall submit to the Food and Drug Administration on or before February 21, 1974, an abbreviated new drug application for these products. Any such drug product then on the market which is not the subject of an application submitted for the drug product shall be subject to regulatory procedures under section 505 of the act. In addition to the information specified in § 314.50 of this chapter, the application shall contain:

(i) A full list of the articles used as components of the digoxin product, specifications for components, detailed identification and analytical procedures used to assure that the components meet established specifications of identity, strength, quality, and purity and a complete description of the manufacturing process.

(ii) The source of the digoxin used in the formulation including the name and address of the supplier.

(iii) A statement that stability studies will be conducted to establish a suitable expiration date for the digoxin product in the form in which it is distributed.

(iv) A statement that the product label will contain a suitable expiration date. In the absence of any stability test data, this expiration date shall be no longer than one year after the batch is manufactured. If the expiration date is greater than one year, supporting stability data shall be included in the application.

(v) Labeling that is in compliance with all requirements of the act and regulations promulgated thereunder, the pertinent parts of which are as indicated in paragraph (e) of this section.

(vi) A statement that the applicant will initiate recall of all stocks of the drug product outstanding when so requested by the Food and Drug Administration.

(vii) A statement that the applicant intends to conduct in vivo bioavailability tests and that the applicant, under the records and reports provisions of section 505(k) of the act, will:

(*a*) Within 30 days after the submission of the application, submit to the Food and Drug Administration the protocol which the applicant proposes to follow in conducting these in vivo bioavailability tests. The protocol shall contain all of the essential elements set forth in paragraph (d) of this section. The tests shall not be initiated prior to receiving notification from the Food and Drug Administration that the bioavailability protocol has been reviewed and either approved or its deficiencies delineated.

(*b*) Within 180 days after receiving notification from the Food and Drug Administration that the bioavailability protocol has been reviewed, submit to the Food and Drug Administration the results of the in vivo bioavailability tests.

(2) Digoxin tablets: Any person marketing digoxin tablets, in addition to complying with all of the requirements of paragraph (a)(1) of this section, shall include in their abbreviated new drug application:

(i) A statement that the applicant will establish procedures to test each lot of digoxin tablets prior to releasing the batch for distribution to assure that the batch meets all of The United States Pharmacopeia (USP XVIII) requirements for digoxin tablets including, but not limited to, potency, content uniformity, and dissolution and either (*a*) that the quantity of digoxin dissolved at one hour is not more than 95 percent of the assayed amount of digoxin or (*b*) that the quantity of digoxin dissolved at 15 minutes is not

more than 90 percent of the assayed amount of digoxin.

(ii) A statement that finished product specifications shall be established to include provisions to assure that the range of average one-hour dissolution values among batches of digoxin tablets does not exceed 20 percent.

(3) Before releasing for distribution any batch of digoxin tablets manufactured after January 22, 1974, the manufacturer shall:

(i) Test a sample of the batch to assure that the batch meets all of the requirements of The United States Pharmacopeia (USP XVIII) including but not limited to, potency, content uniformity, and dissolution and either (*a*) that the quantity of digoxin dissolved at one hour is not more than 95 percent of the assayed amount of digoxin or (*b*) that the quantity of digoxin dissolved at 15 minutes is not more than 90 percent of the assayed amount of digoxin.

(ii) Submit a sample of the batch to the Food and Drug Administration according to the procedures set forth in paragraph (g) of this section. Results of tests conducted on the batch by or for the manufacturer and the batch production record shall accompany the sample.

(iii) Withhold the batch from distribution until he is notified by the Food and Drug Administration that the sample was tested and found to meet all of the requirements in The United States Pharmacopeia (USP XVIII) for potency, content uniformity, and dissolution and either (*a*) that the quantity of digoxin dissolved at one hour is not more than 95 percent of the assayed amount of digoxin or (*b*) that the quantity of digoxin dissolved at 15 minutes is not more than 90 percent of the assayed amount of digoxin.

(iv) Submit a sample of each batch of digoxin tablets as provided for in paragraph (a)(3)(ii) of this section until he is notified by the Food and Drug Administration that he is released from the certification program. This notification will be made on the basis of sample test results, inspectional findings regarding compliance with current good manufacturing practice, and compliance with all other requirements of this section and any other directives issued by the Food and Drug Administration as a condition for release from the certification program.

(4) Any manufacturer who has distributed any batch of digoxin tablets which does not meet the compendial requirement for dissolution, when tested by the method in The United States Pharmacopeia (USP XVIII), shall initiate recall of the subject batch when so requested by the Food and Drug Administration.

(b) Failure of an applicant to submit the protocol and/or the results of the in vivo bioavailability tests showing adequate evidence of the product's bioavailability within the times specified in paragraph (a)(1)(vii) of this section and/or to comply with all of the certification requirements of paragraph (a)(3) of this section shall be justification for withdrawal of approval of the application under section 505(e) of the act.

(c) Any product reformulation or change in manufacturing process will require the submission of a supplement to the approved abbreviated new drug application containing adequate data to demonstrate the bioavailability of the reformulated product. Food and Drug Administration approval of the supplement is required before the reformulated product is marketed. The Food and Drug Administration recommends that, where digoxin tablets are reformulated, manufacturers reformulate their product to achieve dissolution of 70 to 90 percent at one hour when tested by all three methods (i.e., the USP method, and the ''paddle-water'' and ''paddle-acid'' methods) described in paragraph (h) of this section.

(d) The protocol for the in vivo bioavailability tests required in paragraphs (a) and (c) of this section shall employ a three-way crossover design using the digoxin test product; a reference digoxin tablet supplied, on request, by the Food and Drug Administration; and bulk digoxin USP in an oral solution. Appropriate venous blood and urinary samples are to be collected and analyzed. The method shall be capable of detecting the difference between the reference tablet and the reference oral solution. Bioavailability of the test product shall be demonstrated if a mean absorption of at least 75 percent of the combined mean of the two

reference standards is observed. Assistance in developing a protocol for a particular dosage formulation may be obtained by contacting the Food and Drug Administration, Center for Drug Evaluation and Research (HFD–420), 5600 Fishers Lane, Rockville, MD 20857.

(e) Parts of the digoxin product labeling indicated below shall be as follows:

DIGOXIN LABELING GUIDELINES

(ADULT AND PEDIATRIC)

DESCRIPTION

Digoxin is one of the cardiac (or digitalis) glycosides, a closely related group of drugs having in common specific and powerful effects on the myocardium. These drugs are found in a number of plants. The term ''digitalis'' is used to designate the whole group. Typically, the glycosides are composed of three portions: a steroid nucleus, a lactone ring, and a sugar (hence ''glycosides'').

(This section should include a chemical and physical description of digoxin and the same quantitative ingredient information as that required on the label.)

ACTION

The digitalis glycosides have qualitatively the same therapeutic effects on the heart. They (1) increase the force of myocardial contraction, (2) increase the refractory period of the atrioventricular (A–V) node, and (3) to a lesser degree, affect the sinoatrial (S–A) node and conduction system via the parasympathetic and sympathetic nervous systems.

Gastrointestinal absorption of digoxin is a passive process. About 50–75 percent of digoxin in tablet form is absorbed. Digoxin is only 20–25 percent bound to plasma proteins and is predominantly excreted by the kidneys unmetabolized unless there is significant renal failure. Renal excretion of digoxin is proportional to glomerular filtration rate and is largely independent of urine flow. Digoxin is not effectively removed from the body by dialysis, exchange transfusion, or during cardiopulmonary bypass, presumably because of tissue binding. In subjects with normal renal function, digoxin is excreted exponentially with an average half-life of 36 hours, resulting in the loss of 35–40 percent of the body stores daily.

Serum levels and pharmacokinetics are essentially unchanged by massive weight loss, suggesting that lean body mass should be used in dosage calculations. The peak blood level from oral dosing with tablets occurs 1–3 hours after administration. The onset of therapeutic action of digoxin after oral tablets is 1–2 hours, with the peak therapeutic effect occurring 6–8 hours after dosing.

INDICATIONS

1. Congestive heart failure, all degrees, is the primary indication. The increased cardiac output due to digoxin results in diuresis and general amelioration of the disturbances characteristic of right (venous congestion, edema) and left (dyspnea, orthopnea, cardiac asthma) heart failure.

Digoxin, generally, is most effective in ''low output'' failure and less effective in ''high output'' (bronchopulmonary insufficiency, infection, hyperthyroidism) heart failure.

Digoxin should be continued after heart failure is abolished unless some known precipitating factor is corrected.

2. Atrial fibrillation, especially when the ventricular rate is elevated. Digoxin rapidly reduces ventricular rates and eliminates the pulse deficit. Palpitation, precordial distress or weakness are relieved and any concomitant congestive failure ameliorated.

Digoxin should be continued in doses necessary to maintain the desired ventricular rate and other clinical effects.

3. Atrial flutter. Digoxin slows the heart and regular sinus rhythm may appear. Frequently the flutter is converted to atrial fibrillation with a slow ventricular rate. Stopping digoxin at this point may be followed by restoration of sinus rhythm, especially if the flutter was of the paroxysmal type. It is preferable, however, to continue digoxin if failure ensues or if atrial flutter is a frequent occurrence.

4. Paroxysmal atrial tachycardia. Oral digoxin may be used, especially if the condition is resistant to lesser measures. Depending on the urgency, a more rapid acting parenteral preparation may be preferable to initiate digitalization, although if heart failure has ensued or paroxysms recur frequently, digoxin should be maintained by oral administration.

Digoxin is not indicated in sinus tachycardia unless due to heart failure.

5. Cardiogenic shock. The drug is often employed, especially when the condition is accompanied by pulmonary edema. Digoxin seems to affect adversely shock due to septicemia from gram negative bacteria.

CONTRAINDICATIONS

The presence of toxic effects (See ADVERSE REACTIONS section) induced by any digitalis preparation is a contraindication to all of the gylcosides.

Allergy, though rare, does occur. It may not extend to all preparations, and another may be tried.

Ventricular fibrillation.

WARNINGS

Digitalis alone or with other drugs has been promoted for use in the treatment of obesity. This use of digoxin or other

digitalis glycosides is unwarranted. Moreover, since they may cause potentially fatal arrhythmias or other adverse effects, the use of these drugs in the treatment of obesity is dangerous.

Many of the arrhythmias for which digoxin is advised closely resemble those reflecting digoxin intoxication. If the possibility of digoxin intoxication cannot be excluded, cardiac glycosides should be temporarily withheld if permitted by the clinical situation.

The patient with congestive heart failure may complain of nausea and vomiting. These symptoms may also be indications on digoxin intoxication. A clinical determination of the cause of these symptoms must be attempted before further drug administration.

Patients with renal insufficiency require smaller than usual doses of digoxin. See ACTION section for mechanism.

PRECAUTIONS

Atrial arrhythmias associated with hypermetabolic states are particularly resistant to digoxin treatment. Care must be taken to avoid digoxin toxicity if digoxin is used to help the arrhythmia.

Digoxin is not indicated for the treatment of ventricular tachycardia unless congestive heart failure supervenes after a protracted episode not itself due to digoxin.

Potassium depletion sensitizes the myocardium to digoxin, and toxicity may develop even with the usual dosage. Hypokalemia may also alter the rate of onset and intensity of the positive inotropic effect of digoxin. Therefore, it is desirable to maintain normal serum potassium levels in patients being treated with digoxin.

Potassium wastage may result from diuretic or corticosteriod therapy, hemodialysis, and from suction of gastrointestinal secretions. It may accompany malnutrition, diarrhea, prolonged vomiting, old age, and long-standing congestive heart failure. In general, rapid changes in serum potassium or other electrolytes are to be avoided, and intravenous treatment with potassium should be reserved only for special circumstances as described below (see TREATMENT OF ARRHYTHMIAS PRODUCED BY OVERDOSAGES section).

Patients with acute myocardial infarction, severe pulmonary disease, or far advanced heart failure may be more sensitive to digoxin and more prone to disturbances of rhythm.

Calcium affects contractility and excitability of the heart in a manner similar to that of digoxin. Calcium may produce serious arrhythmias in digitalized patients.

In myxedema the digoxin requirements are less because excretion rate is decreased and blood levels are significantly higher.

In incomplete A–V block, especially in patients subject to Stokes-Adams attacks, advanced or complete heart block may develop if digoxin is given. Heart failure in these patients can usually be controlled by other measures and by increasing the heart rate.

Patients with chronic constructive pericarditis may respond unfavorably to digoxin.

Patients with idiopathic hypertrophic subaortic stenosis must be managed extremely carefully. Unless cardiac failure is severe, it is doubtful whether digoxin should be employed.

Renal insufficiency delays the excretion of digoxin, and dosage must be adjusted accordingly in patients with renal disease. NOTE: This applies also to potassium administration should it become necessary.

Electrical conversion of arrhythmias may require reduction of digoxin dosage.

ADVERSE REACTIONS

Gynecomastia, uncommon.

Overdosage or toxic effects.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea are the most common early symptoms of overdosages in the adult (but rarely conspicuous in infants). Uncontrolled heart failure may also produce such symptoms.

Central nervous system: Visual disturbances (blurred vision, yellow vision), headache, weakness, apathy.

Cardiac disturbances (arrhythmias): Ventricular premature beats are the most common, except in infants and young children. Paroxysmal and nonparoxysmal nodal rhythms, atrioventricular (interference) dissociation and paroxysmal atrial tachycardia (PAT) with block are also common arrhythmias due to digoxin overdosage. Conduction disturbances: Excessive slowing of the pulse is a clinical sign of digoxin overdosage. Atrioventricular block of increasing degree may proceed to complete heart block. Note: The electrocardiogram is fundamental in determining the presence and nature of these cardiac toxic disturbances. Digoxin may also induce other changes (as of the ST segment), but these provide no measure of the degree of digitalization.

TREATMENT OF ARRHYTHMIAS PRODUCED BY OVERDOSAGES

Digoxin should be discontinued until all signs of toxicity are abolished. Discontinuation may be all that is necessary if toxic manifestations are not severe and appear after the time for peak effect of the drug.

Potassium salts are commonly used. Potassium chloride in divided oral doses totaling 4–6 grams for adults (see PEDIATRIC INFORMATION section for pediatric dosage) may be given provided renal function is adequate.

When correction of the arrhythmia is urgent and the serum potassium level is low or normal, potassium should be administered

intravenously in a solution of 5 percent dextrose in water. A total of 40–100 milliequivalents (30 milliequivalents per 500 milliliters) is given at the rate of 20 milliequivalents per hour unless limited by pain due to local irritation.

Additional amounts may be given if the arrhythmia is uncontrolled and the potassium well tolerated.

Continuous electrocardiographic monitoring should be performed to watch for any evidence of potassium toxicity, e.g., peaking of T waves, and to observe the effect on the arrhythmia so that the infusion may be promptly stopped when the desired effect is achieved.

CAUTION: Potassium should not be used and may be dangerous for severe or complete heart block due to digoxin and not related to any tachycardia.

Other agents that have been approved for the treatment of digoxin intoxication include procainamide, lidocaine, and propranolol.

DOSAGE AND ADMINISTRATION

Oral digoxin is administered slowly or rapidly as required until the desired therapeutic effect is obtained without symptoms of overdosage. The amount can be predicted approximately from the lean body mass of the patient with allowances made for excretion during the time taken to induce digitalization.

Subsequent maintenance dosage is also determined tentatively by the amount necessary to sustain the desired therapeutic effect.

Recommended dosages are practical average figures that may require considerable modification as dictated by individual sensitivity or associated conditions. Diminished renal function is the most important factor requiring modification of recommended or average doses. (See WARNINGS and PRECAUTIONS sections.)

The average amount of digoxin that patients must accumulate to be digitalized with digoxin tablets is 1.0–1.5 milligrams. Digitalization may be accomplished by any of several approaches that vary in dosage and frequency of administration, but reach the same endpoint in terms of total amount accumulated.

In previously undigitalized patients, a single loading dose of 0.5–0.75 milligram orally usually produces a detectable effect in 1–2 hours that becomes maximal in 6–8 hours. Additional doses of 0.25–0.5 milligram may be given cautiously at 6–8 hour intervals to full digitalization.

In previously undigitalized patients, institution of daily maintenance therapy (0.125–0.5 milligram, see next paragraph) without a loading dose results in development of a steady-state plateau concentrations in about 7 days in patients with normal renal function.

The average daily oral maintenance dose is 0.125–0.5 milligram, usually 0.25 milligram. In the elderly patient, 0.125–0.25 milligram should be considered the average maintenance dose.

In patients with renal impairment, digoxin excretion is impaired and serum half-life is prolonged (see ACTION section). Digitalizing and maintenance doses are lower than those recommended for patients with normal renal functions. Signs of digoxin toxicity develop sooner in patients with renal impairment, and it takes longer for toxic signs and symptoms to disappear. Because of the prolonged half-life, a longer period of time is required to achieve an initial or new steady-state plateau in patients with renal impairment than in patients with normal renal function.

It cannot be overemphasized that the values given are averages and substantial individual variation can be expected.

(If pediatric dosage is available, the labeling sections above should be expanded to include the following information.)

PEDIATRIC INFORMATION

WARNINGS

Newborn infants display considerable variability in their tolerance to digoxin, depending on their degree of maturity.

Premature and immature infants are particularly sensitive, and dosage must be reduced and digitalization should be even more individualized and cautiously approached than in more mature infants. Impaired renal function must also be carefully taken into consideration.

Congestive heart failure accompanying acute glomerulonephritis requires extreme care in digitalization. A relatively low total dose administered in divided doses and concomitant use of antihypertensive drugs has been recommended. ECG monitoring is essential. Digoxin should be discontinued as soon as possible.

Patients with idiopathic hypertrophic subaortic stenosis must be managed extremely carefully. Unless cardiac failure is severe, it is doubtful whether digoxin should be employed.

Patients with rheumatic carditis, especially when severe, are unusually sensitive to digoxin and prone to disturbances of rhythm. If heart failure develops, digitalization may be initiated with relatively low doses; then it can be cautiously increased until a beneficial effect is obtained. If a therapeutic trial does not result in improvement, the drug should be considered ineffective and be discontinued.

NOTE: Digitalis glycosides are an important cause of accidental poisoning in children.

PRECAUTIONS

Dosage must be carefully titrated and differences in the bioavailability of parenteral preparations, elixirs, and tablets should be taken into account when switching patients from one preparation to another.

Electrocardiographic monitoring may be necessary to avoid intoxication.

Premonitory signs of toxicity in the newborn are undue slowing of the sinus rate, sinoatrial arrest, and prolongation of PR interval.

ADVERSE REACTIONS

Toxic signs differ from the adult in a number of respects. Cardiac arrhythmias are the more reliable and frequent signs of toxicity.

Vomiting and diarrhea, neurologic and visual disturbances are rare as initial signs.

Premature ventricular systoles are rarely seen; nodal and atrial systoles are more frequent.

Atrial arrhythmias, atrial ectopic rhythms, and paroxysmal atrial tachycardia with A–V block particularly are more common manifestations of toxicity in children. Ventricular arrhythmias are rare.

TREATMENT OF ARRHYTHMIAS PRODUCED BY OVERDOSAGES

(See adult section for other recommendations for the treatment of arrhythmias produced by overdosages and for additional recommendations and cautions regarding the use of potassium.) Potassium preparations may be given orally in divided doses totaling 1–1.5 milliequivalents/kilogram (1 gram K contains 13.4 milliequivalents). When correction of the arrhythmia is urgent, approximately 0.5 milliequivalents/kilogram of potassium per hour may be given, with careful electrocardiographic monitoring, as a solution of 20 milliequivalents or less per 500 milliliters in 5 percent dextrose in water. The total dose should generally not exceed 2 milliequivalents of potassium/kilogram.

DOSAGE AND ADMINISTRATION

Digitalization must be individualized. Generally, premature and immature infants are particularly sensitive, requiring reduced dosage that must be determined by careful titration.

Oral Dosage. Beyond the immediate newborn period, children require proportionally greater doses than adults on the basis of body weight or surface area. The recommended oral digitalizing dosages in children with normal renal function are:

Newborn infants (normal), up to 1 month, require 40–60 micrograms/kilogram.

Infants, 1 month to 2 years, require approximately 60–80 micrograms/kilogram.

Children 2 years to 10 years, require 40–60 micrograms/kilogram.

Children, over 10 years of age, require adult dosages in proportion to their body weight.

Maintenance therapy is 20–30 percent of the digitalizing dose administered each day.

Long term use of digoxin is indicated in almost all infants who have been digitalized for acute congestive heart failure unless the cause is transient. Many favor maintaining digoxin until at least 2 years of age in all infants with paroxysmal atrial tachycardia or in those who show either definite or latent failure.

Many children with severe inoperable congenital defects need digoxin throughout childhood and often for life.

(f) Abbreviated new drug applications shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Office of Generic Drugs, 5600 Fishers Lane, Rockville, MD 20857.

(g) All samples of digoxin tablets required by paragraph (a)(3) of this section to be submitted to the Food and Drug Administration shall be handled as follows:

(1) The sample shall consist of 6 subsamples of 1000 tablets each collected at random from throughout the manufacturing run. Each of the 6 subsamples shall be identified with the name of the product, the labeled potency, the date of manufacture, the batch number, and the name and address of the manufacturer.

(2) The sample together with the batch production record and results of all tests conducted by or for the manufacturer to determine the product's identity, strength, quality, and purity, content uniformity and dissolution shall be submitted to the Department of Health and Human Services, Public Health Service, FDA National Center for Drug Analysis, 1114 Market St., St. Louis, MO 63101. The outer wrapper shall be identified ''SAMPLE—DIGOXIN CERTIFICATION.''

(h) The Food and Drug Administration is aware of data with two in vitro methods, in addition to that described in The United States Pharmacopeia (USP XVIII), developed to measure digoxin tablets dissolution. These two methods, the so-called ''paddle-water'' and ''paddle-acid'' methods, are described below and are identical with the exception of the nature of the dissolution medium used in the procedures (i.e., distilled or deionized water

vs. dilute hydrochloric acid (0.6 percent volume/volume)). The dissolution apparatus used in these two methods differs significantly from the apparatus described in the method in the compendium. The Food and Drug Administration is aware that the three methods (i.e., USP, ''paddle-water,'' and ''paddle-acid'') show significant differences in dissolution in comparative tests on some formulations. Definitive bioavailability data to compare the relative value of each of these methods to predict bioavailability of the few formulations where the methods show significant differences in dissolution rate are not now available. Manufacturers who conduct research utilizing the ''paddle-water'' and ''paddle-acid'' methods, particularly in comparison with the method in The United States Pharmacopeia, shall submit any data obtained using these methods to the Food and Drug Administration pursuant to section 505(k) of the act.

(1) *Dissolution apparatus.*

(NOTE: Throughout this procedure use scrupulously clean glassware, which previously has been rinsed with dilute hydrochloric acid, distilled or deionized water, then with alcohol, and carefully dried. Take precautions to prevent contamination from airborne, fluorescent particles and from metal and rubber surfaces.) The apparatus consists of a suitable water bath, a 1000 milliliter glass vessel (Kimble Glass No. 26220 or equivalent), a motor, and a polytetrafluoroethylene stirring blade (Sargent S–76637, Size B, 3 inch length; or equivalent) on a glass stirring shaft (Sargent 5–76636, 14.5 inch length; or equivalent). The water bath may be of any convenient size that permits keeping the water temperature uniformly at 37° C. ±0.5° C. throughout the test. The vessel is spherical, and is provided with three ports at the top, one of which is centered. The lower half of the vessel is 65 millimeters in inside radius and the vessel's nominal capacity is 1000 milliliters. The glass stirring shaft from the motor is placed in the center port, and one of the outer ports may be used for insertion of a thermometer. Samples may be removed for analysis through the other port. The motor is fitted with a speed-regulating device that allows the motor speed to be held at 50 rpm ±2 rpm. The motor is suspended above the vessel in such a way that it may be raised or lowered to position the stirring blade. The glass stirring shaft is 10 millimeters in diameter and about 37 centimeters in length. It must run true on the motor axis without perceptible wobble. The polytetrafluoroethylene stirring blade is 4 millimeters thick and forms a section of a circle, whose diameter is 83 millimeters and which is subtended by parallel chords of 42 and 77 millimeters. The blade is positioned horizontally, with the 42-millimeter edge down, 2.5 centimeters ±0.2 centimeter above the lowest inner surface of the vessel.

(2) *Reagents*—(i) *Dissolution medium.* For ''paddle-water,'' use distilled or deionized water. For ''paddle-acid,'' use dilute hydrochloric acid (0.6 percent volume/volume). Use the same batch of dissolution medium throughout the test.

(ii) *Standard solutions.* Accurately weigh approximately 25 milligrams of The United States Pharmacopeia Digoxin Reference Standard, dissolve in a minimum amount of 95 percent ethanol in a 500 milliliter volumetric flask and add 95 percent ethanol to volume and mix. Dilute 10.0 milliliters of this first solution to 100.0 milliliters with 95 percent ethanol and mix for the second solution. Just prior to use, individually dilute 1.0, 2.0, 3.0, 4.0, and 5.0 milliliter aliquots of the second solution with dissolution medium to 50.0 milliliters. These solutions are equivalent to 20, 40, 60, 80, and 100 percent of dissolution, respectively, for a 0.25 milligram digoxin tablet.

(iii) *Extraction solvent.* Prepare a solvent containing 6 volumes of chloroform, analytical reagent grade, with 1 volume of n-propyl alcohol, analytical reagent grade.

(iv) *Ascorbic acid-methanol solution.* Prepare a solution containing 2 milligrams of ascorbic acid, analytical reagent grade, per 1 milliliter of methanol, absolute, analytical reagent grade.

(v) *Hydrochloric acid, concentrated reagent grade.*

(vi) *Hydrogen peroxide-methanol solution.* On the day of use, dilute 2.0 milliliters of recently assayed 30 percent hydrogen peroxide, reagent grade, with methanol, absolute, analytical reagent grade to 100.0 milliliters. Store in a refrigerator. Just prior to use, dilute 2.0 milliliters of this solution with methanol to 100.0 milliliters.

(3) *Procedure*—(i) *Dissolution.* Place 500 milliliters of dissolution medium in the vessel, immerse it in the constant-temperature bath set at 37°C.±0.5°C., and allow the dissolution medium to assume the temperature of the bath.

Position the shaft so that there is a distance of 2.5 centimeters ±0.2 centimeter between the midpoint of the bottom of the blade and the bottom of the vessel. With the stirrer operating at a speed of 50 rpm±2 rpm, place 1 tablet into the flask. After 60 minutes, accurately timed, withdraw 25 milliliters, using a glass syringe connected to a glass sampling tube, of solution from a point midway between the stirring shaft and the wall of the vessel, and approximately midway in depth. Filter the solution promptly after withdrawal, using a suitable membrane filter of not greater than 0.8 micron porosity (Millipore AAWP 025 00, or equivalent), mounted in a suitable holder (Millipore Swinnex SX00 025 00, or equivalent), discarding the first 100 milliliters of filtrate. This is the test solution. Repeat the dissolution procedure on 5 additional tablets.

(ii) *Extraction.* Transfer 10.0 milliliters of each of the six filtrates, 10.0 milliliters of each of the five standard solutions, and 10.0 milliliters of dissolution medium, to provide a blank, in separate 60-milliliter separators. Extract each solution with two 10-milliliter portions of extraction solvent. Combine the extracts of each solution in separate, glass-stoppered, 50-milliliter conical flasks, and evaporate on a steam bath with the aid of a stream of nitrogen to dryness, rinsing the sides of the flasks with extraction solvent. Take care to ensure that all traces of solvent are removed, but avoid prolonged heating. For convenience the residues may be stored in a vacuum desiccator overnight.

(iii) *Measurement of fluorescence.* Begin with the standard solutions, and keep all flasks in the same sequence throughout, so that the elapsed time from addition of reagents to reading of fluorescence is the same for each. Carry the test solutions, standard solutions, and the blank through the determination in one group. Add the following three reagents in as rapid a sequence as possible, swirling after each addition, treating 1 flask at a time, in the order named: 1.0 milliliter of ascorbic acid-methanol solution, 3.0 milliliters of concentrated hydrochloric acid, and 1.0 milliliter of hydrogen peroxide-methanol solution. Insert the stoppers in the flasks, and after 2 hours, measure the fluorescence at about 485 millimicrons, using excitation at about 372 millimicrons. In order to provide a check on the stability of the fluorometer, reread one or more standard solutions. Correct each reading for the blank and plot a standard curve of fluorescence versus precentage dissolution. Determine the percentage dissolution of digoxin in the test solutions by reading from the standard graph.

(iv) Digoxin tablets formulated so that the quantity of digoxin dissolved at one hour, when tested by the method in The United States Pharmacopeia (USP XVIII), is greater than 95 percent of the assayed amount of digoxin and so that the quantity of digoxin dissolved at 15 minutes is greater than 90 percent of the assayed amount of digoxin are new drugs which may be marketed only with an approved full new drug application as provided for in § 314.50 of this chapter. The application shall include, but not be limited to, clinical studies establishing significantly greater bioavailability than digoxin tablets meeting compendial requirements and dosage recommendations based on clinical studies establishing the safe and effective use of the bioavailable digoxin product. Marketing of these digoxin products will be allowed only under a proprietary or trade name, established name, and labeling which differs from that used for digoxin tablets that meet all of the requirements in The United States Pharmacopeia (USP XVIII) and that are formulated so that either (a) the quantity of digoxin dissolved at one hour is not more than 95 percent of the assayed amount of digoxin or (b) the quantity of digoxin dissolved at 15 minutes is not more than 90 percent of the assayed amount of digoxin. New drug applications for these digoxin products shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Office of Drug Evaluation I (HFD–100), 5600 Fishers Lane, Rockville, MD 20857.

[39 FR 11680, Mar. 29, 1974, as amended at 41 FR 43137, Sept. 30, 1976; 41 FR 49482, Nov. 3, 1976; 50 FR 8996, Mar. 6, 1985; 55 FR 11578, Mar. 29, 1990]

§ 310.501 Patient package inserts for oral contraceptives.

(a) *Requirement for a patient package insert.* The safe and effective use of oral contraceptive drug products requires that patients be fully informed of the benefits and the risks involved in their use. An oral contraceptive drug product that does not comply with the requirements of this section is misbranded under section 502 of the Federal Food, Drug, and Cosmetic Act. Each dispenser of an oral contraceptive drug product shall provide a patient package insert to each patient (or to an agent of the patient) to whom the product is dispensed, except that the dispenser may provide the insert to the parent or legal guardian of a legally incompetent patient (or to the agent of either). The patient package insert is required to be placed in or accompany each package dispensed to the patient.

(b) *Distribution requirements.* (1) For oral contraceptive drug products, the manufacturer and distributor shall provide a patient package insert in or with each package of the drug product that the manufacturer or distributor intends to be dispensed to a patient.

(2) Patient package inserts for oral contraceptives dispensed in acute-care hospitals or long-term care facilities will be considered to have been provided in accordance with this section if provided to the patient before administration of the first oral contraceptive and every 30 days thereafter, as long as the therapy continues.

(c) *Contents of patient package insert.* A patient package insert for an oral contraceptive drug product is required to contain the following:

(1) The name of the drug.

(2) A summary including a statement concerning the effectiveness of oral contraceptives in preventing pregnancy, the contraindications to the drug's use, and a statement of the risks and benefits associated with the drug's use.

(3) A statement comparing the effectiveness of oral contraceptives to other methods of contraception.

(4) A boxed warning concerning the increased risks associated with cigarette smoking and oral contraceptive use.

(5) A discussion of the contraindications to use, including information that the patient should provide to the prescriber before taking the drug.

(6) A statement of medical conditions that are not contraindications to use but deserve special consideration in connection with oral contraceptive use and about which the patient should inform the prescriber.

(7) A warning regarding the most serious side effects of oral contraceptives.

(8) A statement of other serious adverse reactions and potential safety hazards that may result from the use of oral contraceptives.

(9) A statement concerning common, but less serious side effects which may help the patient evaluate the benefits and risks from the use of oral contraceptives.

(10) Information on precautions the patients should observe while taking oral contraceptives, including the following:

(i) A statement of risks to the mother and unborn child from the use of oral contraceptives before or during early pregnancy;

(ii) A statement concerning excretion of the drug in human milk and associated risks to the nursing infant;

(iii) A statement about laboratory tests which may be affected by oral contraceptives; and

(iv) A statement that identifies activities and drugs, foods, or other substances the patient should avoid because of their interactions with oral contraceptives.

(11) Information about how to take oral contraceptives properly, including information about what to do if the patient forgets to take the product, information about becoming pregnant after discontinuing use of the drug, a statement that the drug product has been prescribed for the use of the patient and should not be used for other conditions or given to others, and a statement that the patient's pharmacist or practitioner has a more technical leaflet about the drug product that the patient may ask to review.

(12) A statement of the possible benefits associated with oral contraceptive use.

(13) The following information about the drug product and the patient package insert:

(i) The name and place of business of the manufacturer, packer, or distributor, or the name and place of business of the dispenser of the product.

(ii) The date, identified as such, of the most recent revision of the patient package insert placed prominently immediately after the last section of the labeling.

(d) *Other indications.* The patient package insert may identify indications in addition to contraception that are identified in the professional labeling for the drug product.

(e) *Labeling guidance texts.* The Food and Drug Administration issues informal labeling guidance texts under § 10.90(b)(9) of this chapter to provide assistance in meeting the requirements of this section. A request for a copy of the guidance texts should be directed to the Center for Drug Evaluation and Research, Division of Metabolism and Endocrine Drug Products (HFD–510), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(f) *Requirement to supplement approved application.* Holders of approved applications for oral contraceptive drug products that are subject to the requirements of this section are required to submit supplements under § 314.70(c) of this chapter to provide for the labeling required by this section. Such labeling may be put into use without advance approval by the Food and Drug Administration.

[54 FR 22587, May 25, 1989]

§ 310.502 Certain drugs accorded new drug status through rulemaking procedures.

(a) The drugs listed in this paragraph (a) have been determined by rulemaking procedures to be new drugs within the meaning of section 201(p) of the act. Except as provided in paragraph (b) of this section, an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing the following drugs:

(1) Aerosol drug products for human use containing 1,1,1-trichloroethane.

(2) Aerosol drug products containing zirconium.

(3) Amphetamines (amphetamine, dextroamphetamine, and their salts, and levamfetamine and its salts) for human use.

(4) Camphorated oil drug products.

(5) Certain halogenated salicylanilides (tribromsalan (TBS, 3,4′,5-tribromosalicylanilide), dibromsalan (DBS, 4′, 5-dibromosalicylanilide), metabromsalan (MBS, 3, 5-dibromosalicylanilide), and 3,3′, 4,5′-tetrachlorosalicylanilide (TC–SA)) as an ingredient in drug products.

(6) Chloroform used as an ingredient (active or inactive) in drug products.

(7) Cobalt preparations intended for use by man.

(8) Intrauterine devices for human use for the purpose of contraception that incorporate heavy metals, drugs, or other active substances.

(9) Oral prenatal drugs containing fluorides intended for human use.

(10) Parenteral drug products in plastic containers.

(11) Sterilization of drugs by irradiation.

(12) Sweet spirits of nitre drug products.

(13) Thorium dioxide for drug use.

(14) Timed release dosage forms.

(15) Vinyl chloride as an ingredient, including propellant, in aerosol drug products.

(b) Any drug listed in paragraph (a) of this section, when composed wholly or partly of any antibiotic drug, must be certified under section 507 of the act or exempted from certification under section 507 of the act for marketing.

[62 FR 12084, Mar. 14, 1997]

§ 310.503 Requirements regarding certain radioactive drugs.

(a) On January 8, 1963 (28 FR 183), the Commissioner of Food and Drugs exempted investigational radioactive new drugs from part 312 of this chapter provided they were shipped in complete conformity with the regulations issued by the Nuclear Regulatory Commission. This exemption also applied to investigational radioactive biologics.

(b) It is the opinion of the Nuclear Regulatory Commission, and the Food and Drug Administration that this exemption should not apply for certain specific drugs and that these drugs should be appropriately labeled for uses

for which safety and effectiveness can be demonstrated by new-drug applications or through licensing by the Public Health Service in the case of biologics. Continued distribution under the investigational exemption when the drugs are intended for established uses will not be permitted.

(c) Based on its experience in regulating investigational radioactive pharmaceuticals, the Nuclear Regulatory Commission has compiled a list of reactor-produced isotopes for which it considers that applicants may reasonably be expected to submit adequate evidence of safety and effectiveness for use as recommended in appropriate labeling. Such use may include, among others, the uses in this tabulation:

Isotope	Chemical form	Use
Chromium 51	Chromate	Spleen scans.
Do	do	Placenta localization.
Do	do	Red blood cell labeling and survival studies.
Do	Labeled human serum albumin.	Gastrointestinal protein loss studies.
Do	do	Placenta localization.
Do	Labeled red blood cells.	Do.
Cobalt 58 or Cobalt 60.	Labeled cyanocobalamin.	Intestinal absorption studies.
Gold 198	Colloidal	Liver scans.
Do	do	Intracavitary treatment of pleural effusions and/or ascites.
Do	do	Interstitial treatment of cancer.
Iodine 131	Iodide	Diagnosis of thyroid functions.
Do	do	Thyroid scans.
Do	do	Treatment of hyperthyroidism and/or cardiac dysfunction.
Do	do	Treatment of thyroid carcinoma.
Do	Iodinated human serum albumin.	Blood volume determinations.
Do	do	Cisternography.
Do	do	Brain tumor localization.
Do	do	Placenta localization.
Do	do	Cardiac scans for determination of pericardial effusions.
Do	Rose Bengal	Liver function studies.
Do	do	Liver scans.
Do	Iodopyracet, sodium iodohippurate, sodium diatrizoate, diatrizoate methylglucamine, sodium diprotrizoate, sodium acetrizoate, or sodium iothalamate.	Kidney function studies and kidney scans.
Do	Labeled fats and/or fatty acids.	Fat absorption studies.
Do	Cholografin	Cardiac scans for determination of pericardial effusions.
Do	Macroaggregated iodinated human serum albumin.	Lung scans.
Do	Colloidal microaggregated human serum albumin.	Liver scans.
Iodine 125	Iodide	Diagnosis of thyroid function.
Do	Iodinated human serum albumin.	Blood volume determinations.
Do	Rose Bengal	Liver function studies.
Do	Iodopyracet, sodium iodohippurate, sodium diatrizoate, diatrizoate methylglucamine, sodium diprotrizoate, sodium acetrizoate, or sodium iothalamate.	Kidney function studies.
Do	Labeled fats and/or fatty acids.	Fat absorption studies.
Iron 59	Chloride, citrate and/or sulfate.	Iron turnover studies.
Krypton 85	Gas	Diagnosis of cardiac abnormalities.
Mercury 197	Chlormerodrin	Kidney scans.
Do	do	Brain scans.
Mercury 203[1]	do	Kidney scans.
Do	do	Brain scans.
Phosphorus 32	Soluble phosphate	Treatment of polycythemia vera.
Do	do	Treatment of leukemia and bone metastasis.
Do	Colloidal chromic phosphate.	Intracavitary treatment of pleural effusions and/or ascites.
Do	do	Interstitial treatment of cancer.
Potassium 42	Chloride	Potassium space studies.
Selenium 75	Labeled methionine	Pancreas scans.
Strontium 85	Nitrate or chloride	Bone scans on patients with diagnosed cancer.
Technetium 99m.	Pertechnetate	Brain scans.
Do	do	Thyroid scans.
Do	Sulfur colloid	Liver and spleen scans.
Do	Pertechnetate	Placenta localization.
Do	do	Blood pool scans.
Do	do	Salivary gland scans.

Isotope	Chemical form	Use
Do	Diethylenetri-amine pentaacetic acid (DTPA).	Kidney scans.
Xenon 133	Gas	Diagnosis of cardia abnormalities. Cerebral blood-flow studies. Pulmonary function studies. Muscle bloodflow studies.

[1] This item has been removed from the AEC list for kidney scans but is included as the requirements of this order are applicable.

(d)(1) In view of the extent of experience with the isotopes listed in paragraph (c) of this section, the Nuclear Regulatory Commission and the Food and Drug Administration conclude that such isotopes should not be distributed under investigational-use labeling when they are actually intended for use in medical practice.

(2) The exemption referred to in paragraph (a) of this section, as applied to any drug or biologic containing any of the isotopes listed in paragraph (c) of this section, in the ''chemical form'' and intended for the uses stated, is terminated on March 3, 1972, except as provided in paragraph (d)(3) of this section.

(3) The exemption referred to in paragraph (a) of this section, as applied to any drug or biologic containing any of the isotopes listed in paragraph (c) of this section, in the ''chemical form'' and intended for the uses stated, for which drug a new drug application or a ''Investigational New Drug Application'' was submitted prior to March 3, 1972, or for which biologic an application for product license or ''Investigational New Drug Application'' was submitted prior to March 3, 1972, is terminated on August 20, 1976, unless an approvable notice was issued on or before August 20, 1976, in which case the exemption is terminated either upon the subsequent issuance of a nonapprovable notice for the new drug application or on November 20, 1976, whichever occurs first.

(e) No exemption from section 505 of the act or from part 312 of this chapter is in effect or has been in effect for radioactive drugs prepared from accelerator-produced radioisotopes, naturally occurring isotopes, or nonradioactive substances used in conjunction with isotopes.

(f)(1) Based on its experience in regulating investigational radioactive pharmaceuticals, the Nuclear Regulatory Commission has compiled a list of reactor-produced isotopes for which it considers that applicants may reasonably be expected to submit adequate evidence of safety and effectiveness for use as recommended in appropriate labeling; such use may include, among others, the uses in this tabulation:

Isotope	Chemical form	Use
Fluorine 18	Fluoride	Bone imaging.
Indium-113m	Diethylenetriamine pentaacetic acid (DTPA).	Brain imaging; kidney imaging.
Do	Chloride	Placenta imaging; blood pool imaging.
Technetium 99m.	Human serum albumin microspheres.	Lung imaging.
Do	Diethylenetriamine pentaacetic acid (Sn).	Kidney imaging; kidney function studies.
Do	.....do	Brain imaging.
Do	Polyphosphates	Bone imaging.
Do	Technetated aggregated albumin (human).	Lung imaging.
Do	Disodium etidronate	Bone imaging.

(2) In view of the extent of experience with the isotopes listed in paragraph (f)(1) of this section, the Nuclear Regulatory Commission and the Food and Drug Administration conclude that they should not be distributed under investigational-use labeling when they are actually intended for use in medical practice.

(3) Any manufacturer or distributor interested in continuing to ship in interstate commerce drugs containing the isotopes listed in paragraph (f)(1) of this section for any of the indications listed, shall submit, on or before August 25, 1975 to the Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, a new drug application or a ''Investigational New Drug Application'' for each such drug for which the manufacturer or distributor does not have an approved new drug application pursuant to section 505(b) of the act. If the drug is a biologic, a ''Investigational New Drug Application'' or an application for a license under section 351 of the Public Health Service Act shall be submitted to the Center for Biologics Evaluation and

Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20014, in lieu of any submission to the Center for Drug Evaluation and Research.

(4) The exemption referred to in paragraph (a) of this section, as applied to any drug or biologic containing any of the isotopes listed in paragraph (f)(1) of this section, in the ''chemical form'' and intended for the uses stated, is terminated on August 26, 1975 except as provided in paragraph (f)(5) of this section.

(5)(i) Except as provided in paragraph (f)(5)(ii) of this section, the exemption referred to in paragraph (a) of this section, as applied to any drug containing any of the isotopes listed in paragraph (f)(1) of this section, in the ''chemical form'' and intended for the uses stated, for which drug a new drug application or ''Investigational New Drug Application'' was submitted to the Center for Drug Evaluation and Research on or before August 25, 1975 is terminated on August 20, 1976, unless an approvable notice was issued on or before August 20, 1976, in which case the exemption is terminated either upon the subsequent issuance of a nonapprovable notice for the new drug application or on November 20, 1976, whichever occurs first.

(ii) The exemption referred to in paragraph (a) of this section, as applied to any biologic containing any of the isotopes listed in paragraph (f)(1) of this section in the ''chemical form'' and intended for the uses stated, for which biologic an application for product license or ''Investigational New Drug Application'' was submitted to the Center for Biologics Evaluation and Research on or before August 25, 1975 is terminated on October 20, 1976, unless an approvable notice was issued on or before October 20, 1976, in which case the exemption is terminated either upon the subsequent issuance of a nonapprovable notice for the new drug application or on January 20, 1977, whichever occurs first.

(g) The exemption referred to in paragraph (a) of this section, as applied to any drug intended solely for investigational use as part of a research project, which use had been approved on or before July 25, 1975 in accordance with 10 CFR 35.11 (or equivalent regulation of an Agreement State) is terminated on February 20, 1976 if the manufacturer of such drug or the sponsor of the investigation of such drug submits on or before August 25, 1975 to the Food and Drug Administration, Bureau of Drugs, HFD–150, 5600 Fishers Lane, Rockville, MD 20857, the following information:

(1) The research project title;

(2) A brief description of the purpose of the project;

(3) The name of the investigator responsible;

(4) The name and license number of the institution holding the specific license under 10 CFR 35.11 (or equivalent regulation of an Agreement State);

(5) The name and maximum amount per subject of the radionuclide used;

(6) The number of subjects involved; and

(7) The date on which the administration of the radioactive drugs is expected to be completed.

(h) The exemption referred to in paragraph (a) of this section, as applied to any drug not referred to in paragraphs (d), (f), and (g) of this section, is terminated on August 26, 1975.

[39 FR 11680, Mar. 29, 1974, as amended at 40 FR 31307, July 25, 1975; 40 FR 44543, Sept. 29, 1975; 41 FR 35171, Aug. 20, 1976; 41 FR 42947, Sept. 29, 1976; 50 FR 8996, Mar. 6, 1985; 55 FR 11578, Mar. 29, 1990]

§ 310.509 Parenteral drug products in plastic containers.

(a) Any parenteral drug product packaged in a plastic immediate container is not generally recognized as safe and effective, is a new drug within the meaning of section 201(p) of the act, and requires an approved new drug application as a condition for marketing. An ''Investigational New Drug Application'' set forth in part 312 of this chapter is required for clinical investigations designed to obtain evidence of safety and effectiveness.

(b) As used in this section, the term ''large volume parenteral drug product'' means a terminally sterilized aqueous drug product packaged in a single-dose container with a capacity of 100 milliliters or more and intended to be administered or used intravenously in a human.

(c) Until the results of compatibility studies are evaluated, a large volume parenteral drug product for intravenous use in humans that is packaged in a plastic immediate container on or after April 16, 1979, is misbranded unless its labeling contains a warning that includes the following information:

(1) A statement that additives may be incompatible.

(2) A statement that, if additive drugs are introduced into the parenteral system, aseptic techniques should be used and the solution should be thoroughly mixed.

(3) A statement that a solution containing an additive drug should not be stored.

(d) This section does not apply to a biological product licensed under the Public Health Service Act of July 1, 1944 (42 U.S.C. 201).

[62 FR 12084, Mar. 14, 1997]

§ 310.515 Patient package inserts for estrogens.

(a) *Requirement for a patient package insert.* FDA concludes that the safe and effective use of drug products containing estrogens requires that patients be fully informed of the benefits and risks involved in the use of these drugs. Accordingly, except as provided in paragraph (e) of this section, each estrogen drug product restricted to prescription distribution, including products containing estrogens in fixed combinations with other drugs, shall be dispensed to patients with a patient package insert containing information concerning the drug's benefits and risks. An estrogen drug product that does not comply with the requirements of this section is misbranded under section 502(a) of the Federal Food, Drug, and Cosmetic Act.

(b) *Distribution requirements.* (1) For estrogen drug products, the manufacturer and distributor shall provide a patient package insert in or with each package of the drug product that the manufacturer or distributor intends to be dispensed to a patient.

(2) In the case of estrogen drug products in bulk packages intended for multiple dispensing, and in the case of injectables in multiple-dose vials, a sufficient number of patient labeling pieces shall be included in or with each package to assure that one piece can be included with each package or dose dispensed or administered to every patient. Each bulk package shall be labeled with instructions to the dispensor to include one patient labeling piece with each package dispensed or, in the case of injectables, with each dose administered to the patient. This section does not preclude the manufacturer or labeler from distributing additional patient labeling pieces to the dispensor.

(3) Patient package inserts for estrogens dispensed in acute-care hospitals or long-term care facilities will be considered to have been provided in accordance with this section if provided to the patient before administration of the first estrogen and every 30 days thereafter, as long as the therapy continues.

(c) *Patient package insert contents.* A patient package insert for an estrogen drug product is required to contain the following information:

(1) The name of the drug.

(2) The name and place of business of the manufacturer, packer, or distributor.

(3) A statement regarding the benefits and proper uses of estrogens.

(4) The contraindications to use, i.e., when estrogens should not be used.

(5) A description of the most serious risks associated with the use of estrogens.

(6) A brief summary of other side effects of estrogens.

(7) Instructions on how a patient may reduce the risks of estrogen use.

(8) The date, identified as such, of the most recent revision of the patient package insert.

(d) *Guidance language.* The Food and Drug Administration issues informal labeling guidance texts under § 10.90(b)(9) of this chapter to provide assistance in meeting the requirements of paragraph (c) of this section. Requests for a copy of the guidance text should be directed to the Center for Drug Evaluation and Research, Division of Metabolism and Endocrine Drug Products (HFD–510), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(e) *Exemptions.* This section does not apply to estrogen-progestogen oral contraceptives. Labeling requirements for these products are set forth in § 310.501.

(f) *Requirement to supplement approved application.* Holders of approved applications for estrogen drug products that are subject to the requirements of this section must submit supplements under § 314.70(c) of this chapter to provide for the labeling required by paragraph (a) of this section. Such labeling may be put into use without advance approval by the Food and Drug Administration.

[55 FR 18723, May 4, 1990]

§ 310.516 Progestational drug products; labeling directed to the patient.

(a) The Commissioner of Food and Drugs concludes that the safe and effective use of any progestational drug product requires that patients be informed that there is an increased risk of birth defects in children whose mothers have taken this drug during the first 4 months of pregnancy. Accordingly, except as provided by paragraph (d) of this section, any progestational drug product that is the subject of a new drug application approved either before or after October 9, 1962 and all identical, related, or similar drug products as defined in § 310.6, whether or not the subject of an approved new drug application, shall be dispensed to patients with labeling in lay language containing such a warning. The patient labeling shall be provided as a separate printed leaflet independent of any additional materials.

(b) The patient labeling shall specifically include the following:

(1) Name of the drug.

(2) Name and place of business of the manufacturer, packer, or distributor.

(3) A warning that there is an increased risk of birth defects in children whose mothers take this drug during the first 4 months of pregnancy.

(4) A brief discussion of the nature of the risks of birth defects resulting from the use of these drugs during the first 4 months of pregnancy.

(5) A brief statement that these drugs are no longer considered safe as a test for pregnancy.

(6) A statement that the patient should inform her physician as soon as possible if she discovers that she was pregnant when she took the drug.

(c) The patient labeling shall be printed in accordance with the following specifications:

(1) The minimum letter size shall be one-sixteenth of an inch in height.

(2) Letter heights pertain to the lower-case letter ''o'' or its equivalent that shall meet the minumim height standard.

(3) Type used shall conform to the minimum letter height. The body copy shall contain 1-point leading, noncondensed type, and shall not contain any light-face type or small capital letters.

(d) This section does not apply to a progestogen-containing product intended for contraception, which shall be labeled according to the requirements of § 310.501.

(e)(1) Patient labeling for each progestational drug product shall be provided in or with each package intended to be dispensed to the patient. Patient labeling for drug products dispensed in acute-care hospitals or long-term care facilities will be considered to have been provided in accordance with this section if provided to the patient before first administration of the drug and every 30 days thereafter, as long as the therapy continues.

(2) In the case of progestational drug products in bulk packages intended for multiple dispensing, a sufficient number of patient-labeling pieces shall be included in or shall accompany each bulk package to assure that one can be included with each package dispensed to every patient. Each bulk package shall be labeled with instructions to the dispenser to include one patient-labeling piece with each package dispensed to the patient. This section does not preclude the manufacturer or labeler from distributing additional patient-labeling pieces to the dispenser.

(3) In the case of progestational drug products for injection, each package shall include a sufficient number of patient-labeling pieces for the volume of the vial, and instructions to the practitioner administering the drug to give one patient-labeling piece to each premenopausal woman, except those in

whom childbearing is impossible, receiving the drug.

(4) This section does not apply to oral dosage forms labeled solely for the treatment of advanced cancer.

(5) Any progestational drug product, except as noted in paragraphs (d) and (e)(4) of this section, that is not labeled as required by this section and is either introduced or delivered for introduction into interstate commerce, or held for sale after shipment in interstate commerce, is misbranded under section 502 of the Federal Food, Drug, and Cosmetic Act. However, a progestational drug product in the possession of a wholesaler or retailer before December 12, 1978, is not misbranded if adequate numbers of copies of the patient labeling are furnished to the wholesaler or retailer to permit any retail purchaser after that date to obtain such labeling with the product. The requirement that any progestational drug product be dispensed with patient labeling, as applied to physicians who dispense or administer the drug, will not be effective for supplies in their possession on the effective date, but will apply only to supplies received thereafter.

(f) The Food and Drug Administration has available guideline patient labeling for progestational drug products that includes information responsive to all items specified in paragraph (b) of this section. This labeling was published in a separate notice appearing in the FEDERAL REGISTER of January 12, 1989. Any person may rely on this labeling as complying with paragraph (b) of this section.

(g) Holders of approved new drug applications for progestational drug products that are subject to the requirements of this section shall submit supplements under § 314.70(c) of this chapter to provide for the labeling required by paragraph (a) of this section.

[43 FR 47181, Oct. 13, 1978, as amended at 46 FR 53657, Oct. 30, 1981; 54 FR 1163, Jan. 12, 1989]

§ 310.517 Labeling for oral hypoglycemic drugs of the sulfonylurea class.

(a) The University Group Diabetes Program clinical trial has reported an association between the administration of tolbutamide and increased cardiovascular mortality. The Food and Drug Administration has concluded that this reported association provides adequate basis for a warning in the labeling. In view of the similarities in chemical structure and mode of action, the Food and Drug Administration also believes it is prudent from a safety standpoint to consider that the possible increased risk of cardiovascular mortality from tolbutamide applies to all other sulfonylurea drugs as well. Therefore, the labeling for oral hypoglycemic drugs of the sulfonylurea class shall include a warning concerning the possible increased risk of cardiovascular mortality associated with such use, as set forth in paragraph (b) of this section.

(b) Labeling for oral hypoglycemic drugs of the sulfonylurea class shall include in boldface type at the beginning of the ''Warnings'' section of the labeling the following statement:

SPECIAL WARNING ON INCREASED RISK OF CARDIOVASCULAR MORTALITY

The administration of oral hypoglycemic drugs has been reported to be associated with increased cardiovascular mortality as compared to treatment with diet alone or diet plus insulin. This warning is based on the study conducted by the University Group Diabetes Program (UGDP), a long-term prospective clinical trial designed to evaluate the effectiveness of glucose-lowering drugs in preventing or delaying vascular complications in patients with non-insulin-dependent diabetes. The study involved 823 patients who were randomly assigned to one of four treatment groups (*Diabetes*, 19 (supp. 2): 747–830, 1970).

UGDP reported that patients treated for 5 to 8 years with diet plus a fixed dose of tolbutamide (1.5 grams per day) had a rate of cardiovascular mortality approximately 2½ times that of patients treated with diet alone. A significant increase in total mortality was not observed, but the use of tolbutamide was discontinued based on the increase in cardiovascular mortality, thus limiting the opportunity for the study to show an increase in overall mortality. Despite controversy regarding the interpretation of these results, the findings of the UGDP study provide an adequate basis for this warning. The patient should be informed of the potential risks and advantages of (name of drug) and of alternative modes of therapy.

Although only one drug in the sulfonylurea class (tolbutamide) was included in this study, it is prudent from a safety standpoint to consider that this warning may also apply

to other oral hypoglycemic drugs in this class, in view of their close similarities in mode of action and chemical structure.

[49 FR 14331, Apr. 11, 1984]

§ 310.518 Drug products containing iron or iron salts.

Drug products containing elemental iron or iron salts as an active ingredient in solid oral dosage form, e.g., tablets or capsules shall meet the following requirements:

(a) *Packaging.* If the product contains 30 milligrams or more of iron per dosage unit, it shall be packaged in unit-dose packaging. ''Unit-dose packaging'' means a method of packaging a product into a nonreusable container designed to hold a single dosage unit intended for administration directly from that container, irrespective of whether the recommended dose is one or more than one of these units. The term ''dosage unit'' means the individual physical unit of the product, e.g., tablet or capsule. Iron-containing drugs that are subject to this regulation are also subject to child-resistant special packaging requirements in 16 CFR parts 1700, 1701, and 1702.

(b) *Temporary exemption.* (1) Drug products offered in solid oral dosage form (e.g., tablets or capsules), and containing 30 milligrams or more of iron per dosage unit, are exempt from the provisions of paragraph (a) of this section until January 15, 1998, if the sole source of iron in the drug product is carbonyl iron that meets the specifications of § 184.1375 of this chapter.

(2) If this temporary exemption is not extended or made permanent, such drug products shall be in compliance with the provisions of paragraph (a) of this section on or before July 15, 1998.

(c) *Labeling.* (1) The label of any drug in solid oral dosage form (e.g., tablets or capsules) that contains iron or iron salts for use as an iron source shall bear the following statement:

WARNING: Accidental overdose of iron-containing products is a leading cause of fatal poisoning in children under 6. Keep this product out of reach of children. In case of accidental overdose, call a doctor or poison control center immediately.

(2)(i) The warning statement required by paragraph (c)(1) of this section shall appear prominently and conspicuously on the information panel of the immediate container label.

(ii) If a drug product is packaged in unit-dose packaging, and if the immediate container bears labeling but not a label, the warning statement required by paragraph (c)(1) of this section shall appear prominently and conspicuously on the immediate container labeling in a way that maximizes the likelihood that the warning is intact until all of the dosage units to which it applies are used.

(3) Where the immediate container is not the retail package, the warning statement required by paragraph (c)(1) of this section shall also appear prominently and conspicuously on the information panel of the retail package label.

(4) The warning statement shall appear on any labeling that contains warnings.

(5) The warning statement required by paragraph (c)(1) of this section shall be set off in a box by use of hairlines.

(d) The iron-containing inert tablets supplied in monthly packages of oral contraceptives are categorically exempt from the requirements of paragraphs (a) and (c) of this section.

[62 FR 2250, Jan. 15, 1997; 62 FR 15111, Mar. 31, 1997]

§ 310.519 Drug products marketed as over-the-counter (OTC) daytime sedatives.

(a) Antihistamines, bromides, and scopolamine compounds, either singly or in combinations, have been marketed as ingredients in over-the-counter (OTC) drug products for use as daytime sedatives. The following claims have been made for daytime sedative products: ''occasional simple nervous tension,'' ''nervous irritability,'' ''nervous tension headache,'' ''simple nervousness due to common every day overwork and fatigue,'' ''a relaxed feeling,'' ''calming down and relaxing,'' ''gently soothe away the tension,'' ''calmative,'' ''resolving that irritability that ruins your day,'' ''helps you relax,'' ''restlessness,'' ''when you're under occasional stress . . . helps you work relaxed.'' Based on

evidence presently available, there are no ingredients that can be generally recognized as safe and effective for use as OTC daytime sedatives.

(b) Any OTC drug product that is labeled, represented, or promoted as an OTC daytime sedative (or any similar or related indication) is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted as an OTC daytime sedative (or any similar or related indication) is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) Any OTC daytime sedative drug product introduced into interstate commerce after December 24, 1979, that is not in compliance with this section is subject to regulatory action.

[44 FR 36380, June 22, 1979; 45 FR 47422, July 15, 1980, as amended at 55 FR 11579, Mar. 29, 1990]

§ 310.527 Drug products containing active ingredients offered over-the-counter (OTC) for external use as hair growers or for hair loss prevention.

(a) Amino acids, aminobenzoic acid, ascorbic acid, benzoic acid, biotin and all other B-vitamins, dexpanthenol, estradiol and other topical hormones, jojoba oil, lanolin, nucleic acids, polysorbate 20, polysorbate 60, sulfanilamide, sulfur 1 percent on carbon in a fraction of paraffinic hydrocarbons, tetracaine hydrochloride, urea, and wheat germ oil have been marketed as ingredients in OTC drug products for external use as hair growers or for hair loss prevention. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these or any other ingredients intended for OTC external use as a hair grower or for hair loss prevention. Based on evidence currently available, all labeling claims for OTC hair grower and hair loss prevention drug products for external use are either false, misleading, or unsupported by scientific data. Therefore, any OTC drug product for external use containing an ingredient offered for use as a hair grower or for hair loss prevention cannot be considered generally recognized as safe and effective for its intended use.

(b) Any OTC drug product that is labeled, represented, or promoted for external use as a hair grower or for hair loss prevention is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC external use as a hair grower or for hair loss prevention is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After January 8, 1990, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[54 FR 28777, July 7, 1989]

§ 310.528 Drug products containing active ingredients offered over-the-counter (OTC) for use as an aphrodisiac.

(a) Any product that bears labeling claims that it will arouse or increase sexual desire, or that it will improve sexual performance, is an aphrodisiac drug product. Anise, cantharides, don qual, estrogens, fennel, ginseng, golden seal, gotu kola, Korean ginseng, licorice, mandrake, methyltestosterone, minerals, nux vomica, Pega Palo, sarsaparilla, strychnine, testosterone, vitamins, yohimbine, yohimbine hydrochloride, and yohimbinum have been present as ingredients in such drug products. Androgens (e.g., testosterone and methyltestosterone) and estrogens

are powerful hormones when administered internally and are not safe for use except under the supervision of a physician. There is a lack of adequate data to establish general recognition of the safety and effectiveness of any of these ingredients, or any other ingredient, for OTC use as an aphrodisiac. Labeling claims for aphrodisiacs for OTC use are either false, misleading, or unsupported by scientific data. The following claims are examples of some that have been made for aphrodisiac drug products for OTC use: ''acts as an aphrodisiac;'' ''arouses or increases sexual desire and improves sexual performance;'' ''helps restore sexual vigor, potency, and performance;'' ''improves performance, staying power, and sexual potency;'' and ''builds virility and sexual potency.'' Based on evidence currently available, any OTC drug product containing ingredients for use as an aphrodisiac cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or prompted for use as an aphrodisiac is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act, (the act), for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use as an aphrodisiac is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After January 8, 1990, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[54 FR 28786, July 7, 1989]

§ 310.529 Drug products containing active ingredients offered over-the-counter (OTC) for oral use as insect repellents.

(a) Thiamine hydrochloride (vitamin B–1) has been marketed as an ingredient in over-the-counter (OTC) drug products for oral use as an insect repellent (an orally administered drug product intended to keep insects away). There is a lack of adequate data to establish the effectiveness of this, or any other ingredient for OTC oral use as an insect repellent. Labeling claims for OTC orally administered insect repellent drug products are either false, misleading, or unsupported by scientific data. The following claims are examples of some that have been made for orally administered OTC insect repellent drug products: ''Oral mosquito repellent,'' ''mosquitos avoid you,'' ''bugs stay away,'' ''keep mosquitos away for 12 to 24 hours,'' and ''the newest way to fight mosquitos.'' Therefore, any drug product containing ingredients offered for oral use as an insect repellent cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted for oral use as an insect repellent is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug and Cosmetic Act for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted OTC for oral use as an insect repellent is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) Any such drug product in interstate commerce after December 17, 1985, that is not in compliance with

this section is subject to regulatory action.

[40 FR 25171, June 17, 1985, as amended at 55 FR 11579, Mar. 29, 1990]

§ 310.530 Topically applied hormone-containing drug products for over-the-counter (OTC) human use.

(a) The term ''hormone'' is used broadly to describe a chemical substance formed in some organ of the body, such as the adrenal glands or the pituitary, and carried to another organ or tissue, where it has a specific effect. Hormones include, for example, estrogens, progestins, androgens, anabolic steroids, and adrenal corticosteroids, and synthetic analogs. Estrogens, progesterone, pregnenolone, and pregnenolone acetate have been present as ingredients in OTC drug products marketed for topical use as hormone creams. However, there is a lack of adequate data to establish effectiveness for any OTC drug use of these ingredients. Therefore, with the exception of those hormones identified in paragraph (e) of this section, any OTC drug product containing an ingredient offered for use as a topically applied hormone cannot be considered generally recognized as safe and effective for its intended use. The intended use of the product may be inferred from the product's labeling, promotional material, advertising, and any other relevant factor. The use of the word ''hormone'' in the text of the labeling or in the ingredient statement is an implied drug claim. The claim implied by the use of this term is that the product will have a therapeutic or some other physiological effect on the body. Therefore, reference to a product as a ''hormone cream'' or any statement in the labeling indicating that ''hormones'' are present in the product, or any statement that features or emphasizes the presence of a hormone ingredient in the product, will be considered to be a therapeutic claim for the product, or a claim that the product will affect the structure or function of the body, and will consequently cause the product to be a drug.

(b) Any OTC drug product that is labeled, represented, or promoted as a topically applied hormone-containing product for drug use, with the exception of those hormones identified in paragraph (e) of this section, is regarded as a new drug within the meaning of section 201(p) of the act, for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use as a topically applied hormone-containing drug product is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After March 9, 1994, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

(e) This section does not apply to hydrocortisone and hydrocortisone acetate labeled, represented, or promoted for OTC topical use in accordance with part 348 of this chapter.

[58 FR 47610, Sept. 9, 1993]

§ 310.531 Drug products containing active ingredients offered over-the-counter (OTC) for the treatment of boils.

(a) Aminacrine hydrochloride, benzocaine, bismuth subnitrate, calomel, camphor, cholesterol, ergot fluid extract, hexachlorophene, ichthammol, isobutamben, juniper tar (oil of cade), lanolin, magnesium sulfate, menthol, methyl salicylate, oxyguinoline sulfate, petrolatum, phenol, pine tar, rosin, rosin cerate, sassafras oil, sulfur, thymol, triclosan, and zinc oxide have been present in OTC boil treatment drug products. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these or any other ingredient for OTC use for the treatment of boils. Treatment is defined as reducing the size of a boil or reducing an infection related to a boil. Treatment has involved the use of ''drawing salves'' for

these purposes. These ''drawing salves'' contained various ingredients. Based on evidence currently available, any OTC drug product offered for the treatment of boils cannot be considered generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted for the treatment of boils is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any OTC boil treatment drug product is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After May 7, 1991, any such OTC drug product that contains aminacrine hydrochloride, bismuth subnitrate, calomel, camphor, cholesterol, ergot fluid extract, hexachlorophene, isobutamben, juniper tar (oil of cade), lanolin, magnesium sulfate, menthol, methyl salicylate, oxyguinoline sulfate, petrolatum, phenol, pine tar, rosin, rosin cerate, sassafras oil, thymol, or zinc oxide initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

(e) After May 16, 1994, any such OTC drug product that contains benzocaine, ichthammol, sulfur, or triclosan initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

(f) This section does not apply to drug products that contain benzocaine labeled, represented, or promoted for OTC topical use in accordance with part 348 of this chapter.

[58 FR 60336, Nov. 15, 1993]

§ 310.532 Drug products containing active ingredients offered over-the-counter (OTC) to relieve the symptoms of benign prostatic hypertrophy.

(a) The amino acids glycine, alanine, and glutamic acid (alone or in combination) and the ingredient sabal have been present in over-the-counter (OTC) drug products to relieve the symptoms of benign prostatic hypertrophy, e.g., urinary urgency and frequency, excessive urinating at night, and delayed urination. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these or any other ingredients for OTC use in relieving the symptoms of benign prostatic hypertrophy. In addition, there is no definitive evidence that any drug product offered for the relief of the symptoms of benign prostatic hypertrophy would alter the obstructive or inflammatory signs and symptoms of this condition. Therefore, self-medication with OTC drug products might unnecessarily delay diagnosis and treatment of progressive obstruction and secondary infections. Based on evidence currently available, any OTC drug product containing ingredients offered for use in relieving the symptoms of benign prostatic hypertrophy cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted to relieve the symptoms of benign prostatic hypertrophy is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use to relieve the symptoms of benign prostatic hypertrophy is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After August 27, 1990, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[55 FR 6930, Feb. 27, 1990]

§ 310.533 Drug products containing active ingredients offered over-the-counter (OTC) for human use as an anticholinergic in cough-cold drug products.

(a) Atropine sulfate, belladonna alkaloids, and belladonna alkaloids as contained in Atropa belladonna and Datura stramonium have been present as ingredients in cough-cold drug products for use as an anticholinergic. Anticholinergic drugs have been marketed OTC in cough-cold drug products to relieve excessive secretions of the nose and eyes, symptoms that are commonly associated with hay fever, allergy, rhinitis, and the common cold. Atropine sulfate for oral use as an anticholinergic is probably safe at dosages that have been used in marketed cough-cold products (0.2 to 0.3 milligram); however, there are inadequate data to establish general recognition of the effectiveness of this ingredient. The belladonna alkaloids, which contain atropine (*d, dl* hyoscyamine) and scopolamine (*l*- hyoscine), are probably safe for oral use at dosages that have been used in marketed cough-cold products (0.2 milligram) but there are inadequate data to establish general recognition of the effectiveness of these ingredients as an anticholinergic for cough-cold use. Belladonna alkaloids for inhalation use, as contained in Atropa belladonna and Datura stramonium, are neither safe nor effective as an OTC anticholinergic. There are inadequate safety and effectiveness data to establish general recognition of the safety and/or effectiveness or any of these ingredients, or any other ingredient, for OTC use as an anticholinergic in cough-cold drug products.

(b) Any OTC cough-cold drug product that is labeled, represented, or promoted for use as an anticholinergic is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act, for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any cough-cold drug product labeled, represented, or promoted for OTC use as an anticholinergic is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After the effective date of the final regulation, any such OTC cough-cold drug product that is labeled, represented, or promoted for use as an anticholinergic may not be initially introduced or initially delivered for introduction into interstate commerce unless it is the subject of an approved new drug application.

[50 FR 46587, Nov. 8, 1985, as amended at 55 FR 11579, Mar. 29, 1990]

§ 310.534 Drug products containing active ingredients offered over-the-counter (OTC) for human use as oral wound healing agents.

(a) Allantoin, carbamide peroxide in anhydrous glycerin, water soluble chlorophyllins, and hydrogen peroxide in aqueous solution have been present in oral mucosal injury drug products for use as oral wound healing agents. Oral wound healing agents have been marketed as aids in the healing of minor oral wounds by means other than cleansing and irrigating, or by serving as a protectant. Allantoin, carbamide peroxide in anhydrous glycerin, water soluble chlorophyllins, and hydrogen peroxide in aqueous solution are safe for use as oral wound healing agents, but there are inadequate data to establish general recognition of the effectiveness of these ingredients as oral wound healing agents.

(b) Any OTC drug product that is labeled, represented, or promoted for use as an oral wound healing agent is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act, for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for

marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use as an oral wound healing agent is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After the effective date of the final regulation, any OTC drug product that is labeled, represented, or promoted for use as an oral wound healing agent may not be initially introduced or initially delivered for introduction into interstate commerce unless it is the subject of an approved new drug application.

[51 FR 26114, July 18, 1986, as amended at 55 FR 11579, Mar. 29, 1990]

§ 310.536 Drug products containing active ingredients offered over-the-counter (OTC) for use as a nailbiting or thumbsucking deterrent.

(a) Denatonium benzoate and sucrose octaacetate have been present in OTC nailbiting and thumbsucking deterrent drug products. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these and any other ingredients (e.g., cayenne pepper) for OTC use as a nailbiting or thumbsucking deterrent. Based on evidence currently available, any OTC drug product containing ingredients offered for use as a nailbiting or thumbsucking deterrent cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, and promoted as a nailbiting or thumbsucking deterrent is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act) for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use as a nailbiting or thumbsucking deterrent is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After March 2, 1994, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[58 FR 46754, Sept. 2, 1993]

§ 310.537 Drug products containing active ingredients offered over-the-counter (OTC) for oral administration for the treatment of fever blisters and cold sores.

(a) L-lysine (lysine, lysine hydrochloride), *Lactobacillus acidophilus*, and *Lactobacillus bulgaricus* have been present in orally administered OTC drug products to treat fever blisters and cold sores. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these or any other orally administered ingredients for OTC use to treat or relieve the symptoms or discomfort of fever blisters and cold sores. Based on evidence currently available, any OTC drug product for oral administration containing ingredients offered for use in treating or relieving the symptoms or discomfort of fever blisters and cold sores cannot be generally recognized as safe and effective.

(b) Any OTC drug product for oral administration that is labeled, represented, or promoted to treat or relieve the symptoms or discomfort of fever blisters and cold sores is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product for oral administration labeled,

represented, or promoted for OTC use to treat or relieve the symptoms or discomfort of fever blisters and cold sores is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After December 30, 1992, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[57 FR 29173, June 30, 1992]

§ 310.538 Drug products containing active ingredients offered over-the-counter (OTC) for use for ingrown toenail relief.

(a) Any product that bears labeling claims such as for ''temporary relief of discomfort from ingrown toenails,'' or ''ingrown toenail relief product,'' or ''ingrown toenail reliever,'' or similar claims is considered an ingrown toenail relief drug product. Benzocaine, chlorobutanol, chloroxylenol, dibucaine, sodium sulfide, tannic acid, and urea have been present as ingredients in such products. There is lack of adequate data to establish general recognition of the safety and effectiveness of these or any other ingredients for OTC use for ingrown toenail relief. Based on evidence currently available, any OTC drug product containing ingredients offered for use for ingrown toenail relief cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted for ingrown toenail relief is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for ingrown toenail relief is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After March 9, 1994, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[58 FR 47605, Sept. 9, 1993]

§ 310.540 Drug products containing active ingredients offered over-the-counter (OTC) for use as stomach acidifiers.

(a) Betaine hydrochloride, glutamic acid hydrochloride, diluted hydrochloric acid, and pepsin have been present as ingredients in over-the-counter (OTC) drug products for use as stomach acidifiers. Because of the lack of adequate data to establish the effectiveness of these or any other ingredients for use in treating achlorhydria and hypochlorhydria, and because such conditions are asymptomatic, any OTC drug product containing ingredients offered for use as a stomach acidifier cannot be considered generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted for use as a stomach acidifier is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act, for which an approved new drug application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted as a stomach acidifier for OTC use is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After the effective date of the final regulation, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in

compliance with this section is subject to regulatory action.

[53 FR 31271, Aug. 17, 1988]

§ 310.541 Over-the-counter (OTC) drug products containing active ingredients offered for use in the treatment of hypophosphatemia.

(a) Hypophosphatemia is a condition in which an abnormally low plasma level of phosphate occurs in the blood. This condition is not amenable to self-diagnosis or self-treatment. Treatment of this condition should be restricted to the supervision of a physician. For this reason, any drug product containing ingredients offered for OTC use in the treatment of hypophosphatemia cannot be considered generally recognized as safe and effective.

(b) Any drug product that is labeled, represented, or promoted for OTC use in the treatment of hypophosphatemia is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use in the treatment of hypophosphatemia is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of his chapter.

(d) After November 12, 1990, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[55 FR 19858, May 11, 1990]

§ 310.542 Over-the-counter (OTC) drug products containing active ingredients offered for use in the treatment of hyperphosphatemia.

(a) Hyperphosphatemia is a condition in which an abnormally high plasma level of phosphate occurs in the blood. This condition in not amenable to self-diagnosis or self-treatment. Treatment of this condition should be restricted to the supervision of a physician. For this reason, any drug product containing ingredients offered for OTC use in the treatment of hyperphosphatemia cannot be considered generally recognized as safe and effective.

(b) Any drug product that is labeled, represented, or promoted for OTC use in the treatment of hyperphosphatemia is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for use in the treatment of hyperphosphatemia is safe and effective for the purpose intended must comply with the requirements and procedures governing use of investigational new drugs set forth in part 312 of this chapter.

(d) After November 12, 1990, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[55 FR 19858, May 11, 1990]

§ 310.543 Drug products containing active ingredients offered over-the-counter (OTC) for human use in exocrine pancreatic insufficiency.

(a) Hemicellulase, pancreatin, and pancrelipase have been present as ingredients in exocrine pancreatic insufficiency drug products. Pancreatin and pancrelipase are composed of enzymes: amylase, trypsin (protease), and lipase. Significant differences have been shown in the bioavailability of marketed exocrine pancreatic insufficiency drug products produced by different manufacturers. These differences raise a potential for serious risk to patients using these drug products. The bioavailability of pancreatic enzymes is dependent on the process used to manufacture the drug products. Information on this process is not included in an OTC drug monograph. Therefore,

the safe and effective use of these enzymes for treating exocrine pancreatic insufficiency cannot be regulated adequately by an OTC drug monograph. Information on the product's formulation, manufacture, quality control procedures, and final formulation effectiveness testing are necessary in an approved application to ensure that a company has the ability to manufacture a proper bioactive formulation. In addition, continuous physician monitoring of patients who take these drug products is a collateral measure necessary to the safe and effective use of these enzymes, causing such products to be available by prescription only.

(b) Any drug product that is labeled, represented, or promoted for OTC use in the treatment of exocrine pancreatic insufficiency is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use in the treatment of exocrine pancreatic insufficiency is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After May 7, 1991, any such OTC drug product that contains hemicellulase initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

(e) After October 24, 1995, any such OTC drug product that contains pancreatin or pancrelipase initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[60 FR 20165, Apr. 24, 1995]

§ 310.544 Drug products containing active ingredients offered over-the-counter (OTC) for use as a smoking deterrent.

(a) Any product that bears labeling claims that it ''helps stop or reduce the cigarette urge,'' ''helps break the cigarette habit,'' ''helps stop or reduce smoking,'' or similar claims is a smoking deterrent drug product. Cloves, coriander, eucalyptus oil, ginger (Jamaica), lemon oil (terpeneless), licorice root extract, lobeline (in the form of lobeline sulfate or natural lobelia alkaloids or *Lobelia inflata* herb), menthol, methyl salicylate, povidone-silver nitrate, quinine ascorbate, silver acetate, silver nitrate, and thymol have been present as ingredients in such drug products. There is a lack of adequate data to establish general recognition of the safety and effectiveness of these or any other ingredients for OTC use as a smoking deterrent. Based on evidence currently available, any OTC drug product containing ingredients offered for use as a smoking deterrent cannot be generally recognized as safe and effective.

(b) Any OTC drug product that is labeled, represented, or promoted as a smoking deterrent is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use as a smoking deterrent is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After May 7, 1991, any such OTC drug product containing cloves, coriander, eucalyptus oil, ginger (Jamaica), lemon oil (terpeneless), licorice root extract, menthol, methyl salicylate, quinine ascorbate, silver nitrate, and/or thymol initially introduced or

initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action. After December 1, 1993, any such OTC drug product containing lobeline (in the form of lobeline sulfate or natural lobelia alkaloids or *Lobelia inflata* herb), povidone-silver nitrate, silver acetate, or any other ingredients initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[58 FR 31241, June 1, 1993]

§ 310.545 Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses.

(a) A number of active ingredients have been present in OTC drug products for various uses, as described below. However, based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses:

(1) *Topical acne drug products.*

Alcloxa
Alkyl isoquinolinium bromide
Aluminum chlorohydrex
Aluminum hydroxide
Benzocaine
Benzoic acid
Boric acid
Calcium polysulfide
Calcium thiosulfate
Camphor
Chloroxylenol
Cloxyquin
Coal tar
Dibenzothiophene
Estrone
Magnesium aluminum silicate
Magnesium sulfate
Phenol
Phenolate sodium
Phenyl salicylate
Povidone-iodine
Pyrilamine maleate
Resorcinol (as single ingredient)
Resorcinol monoacetate (as single ingredient)
Salicylic acid (over 2 up to 5 percent)
Sodium borate
Sodium thiosulfate
Tetracaine hydrochloride
Thymol
Vitamin E
Zinc oxide
Zinc stearate
Zinc sulfide

(2) *Anticaries drug products*—(i) *Approved as of May 7, 1991.*

Hydrogen fluoride
Sodium carbonate
Sodium monofluorophosphate (6 percent rinse)
Sodium phosphate

(ii) *Approved as of October 7, 1996.*

Calcium sucrose phosphate
Dicalcium phosphate dihydrate
Disodium hydrogen phosphate[1]
Phosphoric acid[1]
Sodium dihydrogen phosphate
Sodium dihydrogen phosphate monohydrate
Sodium phosphate, dibasic anhydrous reagent[1]

(3) *Antidiarrheal drug products.*

Aluminum hydroxide
Atropine sulfate
Calcium carbonate
Carboxymethylcellulose sodium
Glycine
Homatropine methylbromide
Hyoscyamine sulfate
Lactobacillus acidophilus
Lactobacillus bulgaricus
Opium, powdered
Opium tincture
Paregoric
Phenyl salicylate
Scopolamine hydrobromide
Zinc phenolsulfonate

(4) *Antiperspirant drug products.*

Alum, potassium
Aluminum bromohydrate
Aluminum chloride (alcoholic solutions)
Aluminum chloride (aqueous solution) (aerosol only)
Aluminum sulfate
Aluminum sulfate, buffered (aerosol only)
Sodium aluminum chlorohydroxy lactate

(5) [Reserved]

(6) *Cold, cough, allergy, bronchodilator, and antiasthmatic drug products*—(i) *Antihistamine drug products*—(A) *Ingredients.*

Methapyrilene hydrochloride
Methapyrilene fumarate
Thenyldiamine hydrochloride

(B) *Ingredients.*

Phenyltoloxamine dihydrogen citrate
Methapyrilene hydrochloride

[1] These ingredients are nonmonograph except when used to prepare acidulated phosphate fluoride treatment rinses identified in § 355.10(a)(3) of this chapter.

Methapyrilene fumarate
Thenyldiamine hydrochloride

(ii) *Nasal decongestant drug products*—(A) *Approved as of May 7, 1991.*

Allyl isothiocyanate
Camphor (lozenge)
Creosote, beechwood (oral)
Eucalyptol (lozenge)
Eucalyptol (mouthwash)
Eucalyptus oil (lozenge)
Eucalyptus oil (mouthwash)
Menthol (mouthwash)
Peppermint oil (mouthwash)
Thenyldiamine hydrochloride
Thymol
Thymol (lozenge)
Thymol (mouthwash)
Turpentine oil

(B) *Approved as of August 23, 1995.*

Bornyl acetate (topical)
Cedar leaf oil (topical)
Creosote, beechwood (topical)
l-desoxyephedrine (topical)
Ephedrine (oral)
Ephedrine hydrochloride (oral)
Ephedrine sulfate (oral)
Racephedrine hydrochloride (oral/topical)

(iii) *Expectorant drug products.*

Ammonium chloride
Antimony potassium tartrate
Beechwood creosote
Benzoin preparations (compound tincture of benzoin, tincture of benzoin)
Camphor
Chloroform
Eucalyptol/eucalyptus oil
Horehound
Iodides (calcium iodide anyhydrous, hydroidic acid syrup, iodized lime, potassium iodide)
Ipecac
Ipecac fluidextract
Ipecac syrup
Menthol/peppermint oil
Pine tar preparations (extract white pine compound, pine tar, syrup of pine tar, compound white pine syrup, white pine)
Potassium guaiacolsulfonate
Sodium citrate
Squill preparations (squill, squill extract)
Terpin hydrate preparations (terpin hydrate, terpin hydrate elixir)
Tolu preparations (tolu, tolu balsam, tolu balsam tincture)
Turpentine oil (spirits of turpentine)

(iv) *Bronchodilator drug products*—(A) *Approved as of October 2, 1987.*

Aminophylline
Belladonna alkaloids
Euphorbia pilulifera
Metaproterenol sulfate
Methoxyphenamine hydrochloride
Pseudoephedrine hydrochloride
Pseudoephedrine sulfate
Theophylline, anhydrous
Theophylline calcium salicylate
Theophylline sodium glycinate

(B) Approved as of January 29, 1996. Any combination drug product containing theophylline (e.g., theophylline and ephedrine, or theophylline and ephedrine and phenobarbital).

(C) Approved as of June 19, 1996. Any ingredient(s) in a pressurized metered-dose inhaler container.

(7) *Dandruff/seborrheic dermatitis/psoriasis drug products.*

Alkyl isoquinolinium bromide
Allantoin
Benzalkonium chloride
Benzethonium chloride
Boric acid
Calcium undecylenate
Captan
Chloroxylenol
Colloidal oatmeal
Cresol, saponated
Ethohexadiol
Eucalyptol
Juniper tar
Lauryl isoquinolinium bromide
Menthol
Mercury oleate
Methylbenzethonium chloride
Methyl salicylate
Phenol
Phenolate sodium
Pine tar
Povidone-iodine
Resorcinol
Sodium borate
Sodium salicylate
Thymol
Undecylenic acid

(8) *Digestive aid drug products*—(i) *Approved as of May 7, 1991.*

Bismuth sodium tartrate
Calcium carbonate
Cellulase
Dehydrocholic acid
Dihydroxyaluminum sodium carbonate
Duodenal substance
Garlic, dehydrated
Glutamic acid hydrochloride
Hemicellulase
Homatropine methylbromide
Magnesium hydroxide
Magnesium trisilicate
Ox bile extract
Pancreatin
Pancrelipase
Papain
Peppermint oil
Pepsin
Sodium bicarbonate

Sodium citrate
Sorbitol

(ii) *Approved as of November 10, 1993.*

Alcohol
Aluminum hydroxide
Amylase
Anise seed
Aromatic powder
Asafetida
Aspergillus oryza enzymes (except lactase enzyme derived from *Aspergillus oryzae*)
Bacillus acidophilus
Bean
Belladonna alkaloids
Belladonna leaves, powdered extract
Betaine hydrochloride
Bismuth subcarbonate
Bismuth subgallate
Black radish powder
Blessed thistle (cnicus benedictus)
Buckthorn
Calcium gluconate
Capsicum
Capsicum, fluid extract of
Carbon
Cascara sagrada extract
Catechu, tincture
Catnip
Chamomile flowers
Charcoal, wood
Chloroform
Cinnamon oil
Cinnamon tincture
Citrus pectin
Diastase
Diastase malt
Dog grass
Elecampane
Ether
Fennel acid
Galega
Ginger
Glycine
Hydrastis canadensis (golden seal)
Hectorite
Horsetail
Huckleberry
Hydrastis fluid extract
Hydrochloric acid
Iodine
Iron ox bile
Johnswort
Juniper
Kaolin, colloidal
Knotgrass
Lactic acid
Lactose
Lavender compound, tincture of
Linden
Lipase
Lysine hydrochloride
Mannitol
Mycozyme
Myrrh, fluid extract of
Nettle
Nickel-pectin
Nux vomica extract
Orthophosphoric acid
Papaya, natural
Pectin
Peppermint
Peppermint spirit
Phenacetin
Potassium bicarbonate
Potassium carbonate
Protease
Prolase
Rhubarb fluid extract
Senna
Sodium chloride
Sodium salicylate
Stem bromelain
Strawberry
Strychnine
Tannic acid
Trillium
Woodruff

(iii) Charcoal, activated

(9) [Reserved]

(10) *External analgesic drug products*—(i) *Analgesic and anesthetic drug products.*

Aspirin
Chloral hydrate
Chlorobutanol
Cyclomethycaine sulfate
Eugenol
Hexylresorcinol
Methapyrilene hydrochloride
Salicylamide
Thymol

(ii) *Counterirritant drug products.*

Chloral hydrate
Eucalyptus oil

(iii) *Male genital desensitizer drug products.*

Benzyl alcohol
Camphorated metacresol
Ephedrine hydrochloride

(iv) *Diaper rash drug products.*

Any ingredient(s) labeled with claims or directions for use in the treatment and/or prevention of diaper rash.

(v) *Fever blister and cold sore treatment drug products.*

Allyl isothiocyanate
Aspirin
Bismuth sodium tartrate
Camphor (exceeding 3 percent)
Capsaicin
Capsicum
Capsicum oleoresin
Chloral hydrate
Chlorobutanol
Cyclomethycaine sulfate
Eucalyptus oil
Eugenol

Glycol salicylate
Hexylresorcinol
Histamine dihydrochloride
Menthol (exceeding 1 percent)
Methapyrilene hydrochloride
Methyl nicotinate
Methyl salicylate
Pectin
Salicylamide
Strong ammonia solution
Tannic acid
Thymol
Tripelennamine hydrochloride
Trolamine salicylate
Turpentine oil
Zinc sulfate

(vi) *Insect bite and sting drug products.*

Alcohol
Alcohol, ethoxylated alkyl
Benzalkonium chloride
Calamine
Ergot fluidextract
Ferric chloride
Panthenol
Peppermint oil
Pyrilamine maleate
Sodium borate
Trolamine salicylate
Turpentine oil
Zinc oxide
Zirconium oxide

(vii) *Poison ivy, poison oak, and poison sumac drug products.*

Alcohol
Aspirin
Benzethonium chloride
Benzocaine (0.5 to 1.25 percent)
Bithionol
Calamine
Cetalkonium chloride
Chloral hydrate
Chlorobutanol
Chlorpheniramine maleate
Creosote, beechwood
Cyclomethycaine sulfate
Dexpanthenol
Diperodon hydrochloride
Eucalyptus oil
Eugenol
Glycerin
Glycol salicylate
Hectorite
Hexylresorcinol
Hydrogen peroxide
Impatiens biflora tincture
Iron oxide
Isopropyl alcohol
Lanolin
Lead acetate
Merbromin
Mercuric chloride
Methapyrilene hydrochloride
Panthenol
Parethoxycaine hydrochloride
Phenyltoloxamine dihydrogen citrate
Povidone-vinylacetate copolymers
Pyrilamine maleate
Salicylamide
Salicylic acid
Simethicone
Sulfur
Tannic acid
Thymol
Trolamine salicylate
Turpentine oil
Zirconium oxide
Zyloxin

(11) [Reserved]

(12) *Laxative drug products*—(i) *Bulk laxatives.*

Agar
Carrageenan (degraded)
Carrageenan (native)
Guar gun

(ii) *Saline laxative.*

Tartaric acid

(iii) *Stool softener.*

Poloxamer 188

(iv) *Stimulant laxatives.*

Aloin
Bile salts/acids
Calcium pantothenate
Calomel
Colocynth
Elaterin resin
Frangula
Gamboge
Ipomea
Jalap
Ox bile
Podophyllum resin
Prune concentrate dehydrate
Prune powder
Rhubarb, Chinese
Sodium Oleate

(13) [Reserved]

(14) *Oral health care drug products (nonantimicrobial).*

Antipyrine
Camphor
Cresol
Dibucaine
Dibucaine hydrochloride
Eucalyptol
Lidocaine
Lidocaine hydrochloride
Methly salicylate
Myrrh tincture
Pyrilamine maleate
Sorbitol
Sugars
Tetracaine
Tetracaine hydrochloride
Thymol

(15) *Topical otic drug products for the prevention of swimmer's ear and for the drying of water-clogged ears*—(i) *Approved as of May 7, 1991.*

Acetic acid

(ii) *Approved as of August 15, 1995.*

Glycerin and anhydrous glycerin
Isopropyl alcohol

(16) *Poison treatment drug products.*

Ipecac fluidextract
Ipecac tincture
Zinc sulfate

(17) *Skin bleaching drug products.*

Mercury, ammoniated

(18) *Skin protectant drug products.* (i) Ingredients.

Allantoin (wound healing claims only)
Sulfur
Tannic acid
Zinc acetate (wound healing claims only)

(ii) *Astringent drug products.*

Acetone
Alcohol
Alum, ammonium
Alum, potassium
Aluminum chlorhydroxy complex
Aromatics
Benzalkonium chloride
Benzethonium chloride
Benzocaine
Benzoic acid
Boric acid
Calcium acetate
Camphor gum
Clove oil
Colloidal oatmeal
Cresol
Cupric sulfate
Eucalyptus oil
Eugenol
Ferric subsulfate (Monsel's Solution)
Honey
Isopropyl alcohol
Menthol
Methyl salicylate
Oxyquinoline sulfate
P-t-butyl-m-cresol
Peppermint oil
Phenol
Polyoxeythylene laurate
Potassium ferrocyanide
Sage oil
Silver nitrate
Sodium borate
Sodium diacetate
Talc
Tannic acid glycerite
Thymol
Topical starch
Zinc chloride
Zinc oxide
Zinc phenolsulfonate
Zinc stearate
Zinc sulfate

(iii) *Diaper rash drug products.*

Aluminum hydroxide
Cocoa butter
Cysteine hydrochloride
Glycerin
Protein hydrolysate
Racemethionine
Sulfur
Tannic acid
Zinc acetate
Zinc carbonate

(iv) *Fever blister and cold sore treatment drug products.*

Bismuth subnitrate
Boric acid
Pyridoxine hydrochloride
Sulfur
Tannic acid
Topical starch
Trolamine
Zinc sulfate

(v) *Insect bite and sting drug products.*

Alcohol
Alcohol, ethoxylated alkyl
Ammonia solution, strong
Ammonium hydroxide
Benzalkonium chloride
Camphor
Ergot fluidextract
Ferric chloride
Menthol
Peppermint oil
Phenol
Pyrilamine maleate
Sodium borate
Trolamine
Turpentine oil
Zirconium oxide

(vi) *Poison ivy, poison oak, and poison sumac drug products.*

Alcohol
Anion and cation exchange resins buffered
Benzethonium chloride
Benzocaine
Benzyl alcohol
Bismuth subnitrate
Bithionol
Boric acid
Camphor
Cetalkonium chloride
Chloral hydrate
Chlorpheniramine maleate
Creosote
Diperodon hydrochloride
Diphenhydramine hydrochloride
Eucalyptus oil
Ferric chloride

Glycerin
Hectorite
Hydrogen peroxide
Impatiens biflora tincture
Iron oxide
Isopropyl alcohol
Lanolin
Lead acetate
Lidocaine
Menthol
Merbromin
Mercuric chloride
Panthenol
Parethoxycaine hydrochloride
Phenol
Phenyltoloxamine dihydrogen citrate
Povidone-vinylacetate copolymers
Salicylic acid
Simethicone
Tannic acid
Topical starch
Trolamine
Turpentine oil
Zirconium oxide
Zyloxin

(19) [Reserved]

(20) *Weight control drug products.*

Alcohol
Alfalfa
Alginic acid
Anise oil
Arginine
Ascorbic acid
Bearberry
Biotin
Bone marrow, red
Buchu
Buchu, potassium extract
Caffeine
Caffeine citrate
Calcium
Calcium carbonate
Calcium caseinate
Calcium lactate
Calcium pantothenate
Carboxymethylcellulose sodium
Carrageenan
Cholecalcierol
Choline
Chondrus
Citric acid
Cnicus benedictus
Copper
Copper gluconate
Corn oil
Corn syrup
Corn silk, potassium extract
Cupric sulfate
Cyanocobalamin (vitamin B_{12})
Cystine
Dextrose
Docusate sodium
Ergocalciferol
Ferric ammonium citrate
Ferric pyrophosphate
Ferrous fumarate
Ferrous gluconate
Ferrous sulfate (iron)
Flax seed
Folic acid
Fructose
Guar gum
Histidine
Hydrastis canadensis
Inositol
Iodine
Isoleucine
Juniper, potassium extract
Karaya gum
Kelp
Lactose
Lecithin
Leucine
Liver concentrate
Lysine
Lysine hydrochloride
Magnesium
Magnesium oxide
Malt
Maltodextrin
Manganese citrate
Mannitol
Methionine
Methylcellulose
Mono- and di-glycerides
Niacinamide
Organic vegetables
Pancreatin
Pantothenic acid
Papain
Papaya enzymes
Pepsin
Phenacetin
Phenylalanine
Phosphorus
Phytolacca
Pineapple enzymes
Plantago seed
Potassium citrate
Pyridoxine hydrochloride (vitamin B_6)
Riboflavin
Rice polishings
Saccharin
Sea minerals
Sesame seed
Sodium
Sodium bicarbonate
Sodium caseinate
Sodium chloride (salt)
Soybean protein
Soy meal
Sucrose
Thiamine hydrochloride (vitamin B_1)
Thiamine mononitrate (vitamin B_1 mononitrate)
Threonine
Tricalcium phosphate
Tryptophan
Tyrosine
Uva ursi, potassium extract
Valine
Vegetable

Vitamin A
Vitamin A acetate
Vitamin A palmitate
Vitamin E
Wheat germ
Xanthan gum
Yeast

(21) *Ophthalmic drug products.*

(i) *Ophthalmic anesthetic drug products.*

Antipyrine
Piperocaine hydrochloride

(ii) *Ophthalmic anti-infective drug products.*

Boric acid
Mild silver protein
Yellow mercuric oxide

(iii) *Ophthalmic astringent drug products.*

Infusion of rose petals

(iv) *Ophthalmic demulcent drug products.*

Polyethylene glycol 6000

(v) *Ophthalmic vasoconstrictor drug products.*

Phenylephrine hydrochloride (less than 0.08 percent)

(22) *Topical antifungal drug products.*

(i) *Diaper rash drug products.* Any ingredient(s) labeled with claims or directions for use in the treatment and/or prevention of diaper rash.

(ii) *Ingredients.*

Alcloxa
Alum, potassium
Aluminum sulfate
Amyltricresols, secondary
Basic fuchsin
Benzethonium chloride
Benzoic acid
Benzoxiquine
Boric acid
Camphor
Candicidin
Chlorothymol
Coal tar
Dichlorophen
Menthol
Methylparaben
Oxyquinoline
Oxyquinoline sulfate
Phenol
Phenolate sodium
Phenyl salicylate
Propionic acid
Propylparaben
Resorcinol
Salicylic acid
Sodium borate
Sodium caprylate
Sodium propionate
Sulfur
Tannic acid
Thymol
Tolindate
Triacetin
Zinc caprylate
Zinc propionate

(iii) Any ingredient(s) labeled with claims or directions for use on the scalp or on the nails.

(iv) *Ingredients.*

Camphorated metacresol
Chloroxylenol
m-cresol
Nystatin

(23) *Internal analgesic drug products.*

Aminobenzoic acid
Antipyrine
Aspirin, aluminum
Calcium salicylate
Codeine
Codeine phosphate
Codeine sulfate
Iodoantipyrine
Lysine aspirin
Methapyrilene fumarate
Phenacetin
Pheniramine maleate
Pyrilamine maleate
Quinine
Salsalate
Sodium aminobenzoate

(24) *Orally administered menstrual drug products.*

Alcohol
Alfalfa leaves
Aloes
Asclepias tuberosa
Asparagus
Barosma
Bearberry (extract of uva ursi)
Bearberry fluidextract (extract of bearberry)
Blessed thistle (cnicus benedictus)
Buchu powdered extract (extract of buchu)
Calcium lactate
Calcium pantothenate
Capsicum oleoresin
Cascara fluidextract, aromatic (extract of cascara)
Chlorprophenpyridamine maleate
Cimicifuga racemosa
Codeine
Collinsonia (extract stone root)
Corn silk
Couch grass
Dog grass extract
Ethyl nitrite
Ferric chloride
Ferrous sulfate
Gentiana lutea (gentian)

Glycyrrhiza (licorice)
Homatropine methylbromide
Hydrangea, powdered extract (extract of hydrangea)
Hydrastis canadensis (golden seal)
Hyoscyamine sulfate
Juniper oil (oil of juniper)
Magnesium sulfate
Methapyrilene hydrochloride
Methenamine
Methylene blue
Natural estrogenic hormone
Niacinamide
Nutmeg oil (oil of nutmeg)
Oil of erigeron
Parsley
Peppermint spirit
Pepsin, essence
Phenacetin
Phenindamine tartrate
Phenyl salicylate
Piscidia erythrina
Pipsissewa
Potassium acetate
Potassium nitrate
Riboflavin
Saw palmetto
Senecio aureus
Sodium benzoate
Sodium nitrate
Sucrose
Sulferated oils of turpentine
Taraxacum officinale
Theobromine sodium salicylate
Theophylline
Thiamine hydrochloride
Triticum
Turpentine, venice (venice turpertine)
Urea

(25) *Pediculicide drug products*—(i) *Approved as of November 10, 1993.*

Benzocaine
Benzyl alcohol
Benzyl benzoate
Chlorophenothane (dichlorodiphenyl trichloroethane)
Coconut oil soap, aqueous
Copper oleate
Docusate sodium
Formic acid
Isobornyl thiocyanoacetate
Picrotoxin
Propylene glycol
Sabadilla alkaloids
Sulfur, sublimed
Thiocyanoacetate

(ii) *Approved as of June 14, 1994.* The combination of pyrethrum extract (formerly named pyrethrins) and piperonyl butoxide in an aerosol dosage formulation.

(26) *Anorectal druq products*—(i) *Anticholinergic drug products.*

Atropine
Belladonna extract

(ii) *Antiseptic drug products.*

Boric acid
Boroglycerin
Hydrastis
Phenol
Resorcinol
Sodium salicylic acid phenolate

(iii) *Astringent drug products.*

Tannic acid

(iv) *Counterirritant drug products.*

Camphor (greater than 3 to 11 percent)
Hydrastis
Menthol (1.25 to 16 percent)
Turpentine oil (rectified) (6 to 50 percent)

(v) *Keratolytic drug products.*

Precipitated sulfur
Sublimed sulfur

(vi) *Local anesthetic drug products.*

Diperodon
Phenacaine hydrochloride

(vii) *Other druq products.*

Collinsonia extract
Escherichia coli vaccines
Lappa extract
Leptandra extract
Live yeast cell derivative
Mullein

(viii) *Protectant druq products.*

Bismuth oxide
Bismuth subcarbonate
Bismuth subgallate
Bismuth subnitrate
Lanolin alcohols

(ix) *Vasoconstrictor druq products.*

Epinephrine undecylenate

(x) *Wound healinq druq products.*

Cholecalciferol
Cod liver oil
Live yeast cell derivative
Peruvian balsam
Shark liver oil
Vitamin A

(b) Any OTC drug product that is labeled, represented, or promoted for the uses specified and containing any active ingredient(s) as specified in paragraph (a) of this section is regarded as a new drug within the meaning of section 210(p) of the Federal Food, Drug, and Cosmetic Act (the Act), for which an approved new drug application under section 505 of the Act and part

314 of this chapter is required for marketing. In the absence of an approved new drug application, such product is also misbranded under section 502 of the Act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for the OTC uses and containing any active ingredient(s) as specified in paragraph (a) of this section is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) Any OTC drug product that is not in compliance with this section is subject to regulatory action if initially introduced or initially delivered for introduction into interstate commerce after the dates specified in paragraphs (d)(1) through (d)(25) of this section.

(1) May 7, 1991, for products subject to paragraphs (a)(1) through (a)(2)(i), (a)(3) through (a)(4), (a)(6)(i)(A), (a)(6)(ii)(A), (a)(7) (except as covered by paragraph (d)(3) of this section), (a)(8)(i), (a)(9) through (a)(10)(iii), (a)(12)(i) through (a)(12)(iv), (a)(14) through (a)(15)(i), and (a)(16) through (a)(18)(i) of this section.

(2) February 10, 1992, for products subject to paragraph (a)(20) of this section.

(3) December 4, 1992, for products subject to paragraph (a)(7) of this section that contain menthol as an antipruritic in combination with the antidandruff ingredient coal tar identified in § 358.710(a)(1) of this chapter.

(4) February 28, 1990, for products subject to paragraph (a)(6)(iii) of this section, except those that contain ipecac.

(5) September 14, 1993, for products subject to paragraph (a)(6)(iii) of this section that contain ipecac.

(6) December 9, 1993, for products subject to paragraph (a)(6)(i)(B) of this section.

(7) March 6, 1989, for products subject to paragraph (a)(21) of this section, except those that contain ophthalmic anti–infective ingredients listed in paragraph (a)(21)(ii).

(8) June 18, 1993, for products subject to paragraph (a)(21) of this section that contain ophthalmic anti–infective ingredients.

(9) June 18, 1993, for products subject to paragraph (a)(10)(iv) of this section.

(10) June 18, 1993, for products subject to paragraph (a)(22)(i) of this section.

(11) November 10, 1993, for products subject to paragraph (a)(18)(ii) of this section, except products that contain ferric subsulfate.

(12) March 2, 1994, for products subject to paragraph (a)(22)(iii) of this section.

(13) August 5, 1991, for products subject to paragraphs (a)(26) of this section, except for those that contain live yeast cell derivative.

(14) September 2, 1994, for products subject to paragraph (a)(26)(vii) and (a)(26)(x) of this section that contain live yeast cell derivative.

(15) September 23, 1994, for products subject to paragraph (a)(22)(iv) of this section.

(16) June 14, 1994, for products subject to paragraph (a)(25)(ii) of this section.

(17) [Reserved]

(18) August 15, 1995, for products subject to paragraph (a)(15)(ii) of this section.

(19) October 2, 1987, for products subject to paragraph (a)(6)(iv)(A) of this section.

(20) January 29, 1996, for products subject to paragraph (a)(6)(iv)(B) of this section.

(21) April 21, 1994, for products subject to paragraph (a)(8)(iii) of this section.

(22) April 21, 1993, for products subject to paragraph (a)(18)(ii) of this section that contain ferric subsulfate.

(23) August 23, 1995, for products subject to paragraph (a)(6)(ii)(B) of this section.

(24) October 7, 1996, for products subject to paragraph (a)(2)(ii) of this section.

(25) June 19, 1996, for products subject to paragraph (a)(6)(iv)(C) of this section.

[55 FR 46919, Nov. 7, 1990]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 310.545, see the List of CFR Sections Affected in the Finding Aids section of this volume.

EFFECTIVE DATE NOTES: 1. At 60 FR 42436, Aug. 16, 1995, in § 310.545, paragraph (a)(15)(ii)

was stayed for topical otic drug products for the drying of water-clogged ears.

2. At 61 FR 9571, Mar. 8, 1996, in § 310.545 in paragraph (a)(6)(ii)(B), the entry for ''l-desoxyephedrine (topical)'' was stayed until further notice.

§ 310.546 Drug products containing active ingredients offered over-the-counter (OTC) for the treatment and/or prevention of nocturnal leg muscle cramps.

(a) Quinine sulfate alone or in combination with vitamin E has been present in over-the-counter (OTC) drug products for the treatment and/or prevention of nocturnal leg muscle cramps, i.e., a condition of localized pain in the lower extremities usually occurring in middle life and beyond with no regular pattern concerning time or severity. There is a lack of adequate data to establish general recognition of the safety and effectiveness of quinine sulfate, vitamin E, or any other ingredients for OTC use in the treatment and/or prevention of nocturnal leg muscle cramps. In the doses used to treat or prevent this condition, quinine sulfate has caused adverse events such as transient visual and auditory disturbances, dizziness, fever, nausea, vomiting, and diarrhea. Quinine sulfate may cause unpredictable serious and life-threatening hypersensitivity reactions requiring medical intervention and hospitalization; fatalities have been reported. The risk associated with use of quinine sulfate, in the absence of evidence of its effectiveness, outweighs any potential benefit in treating and/or preventing this benign, self-limiting condition. Based upon the adverse benefit-to-risk ratio, any drug product containing quinine or quinine sulfate cannot be considered generally recognized as safe for the treatment and/or prevention of nocturnal leg muscle cramps.

(b) Any OTC drug product that is labeled, represented, or promoted for the treatment and/or prevention of nocturnal leg muscle cramps is regarded as a new drug within the meaning of section 201(p) of the Federal Food, Drug, and Cosmetic Act (the act), for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of nocturnal leg muscle cramps is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After February 22, 1995, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[59 FR 43252, Aug. 22, 1994]

§ 310.547 Drug products containing quinine offered over-the-counter (OTC) for the treatment and/or prevention of malaria.

(a) Quinine and quinine salts have been used OTC for the treatment and/or prevention of malaria, a serious and potentially life-threatening disease. Quinine is no longer the drug of choice for the treatment and/or prevention of most types of malaria. In addition, there are serious and complicating aspects of the disease itself and some potentially serious and life-threatening risks associated with the use of quinine at doses employed for the treatment of malaria. There is a lack of adequate data to establish general recognition of the safety of quinine drug products for OTC use in the treatment and/or prevention of malaria. Therefore, quinine or quinine salts cannot be safely and effectively used for the treatment and/or prevention of malaria except under the care and supervision of a doctor.

(b) Any OTC drug product containing quinine or quinine salts that is labeled, represented, or promoted for the treatment and/or prevention of malaria is regarded as a new drug within the meaning of section 201(p) of the act, for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter

is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of malaria is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After April 20, 1998, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[63 FR 13528, Mar. 20, 1998]

EFFECTIVE DATE NOTE: At 63 FR 13528, Mar. 20, 1998, §310.547 was added to subpart E, effective Apr. 20, 1998.

PART 312—INVESTIGATIONAL NEW DRUG APPLICATION

Subpart A—General Provisions

Sec.
312.1 Scope.
312.2 Applicability.
312.3 Definitions and interpretations.
312.6 Labeling of an investigational new drug.
312.7 Promotion and charging for investigational drugs.
312.10 Waivers.

Subpart B—Investigational New Drug Application (IND)

312.20 Requirement for an IND.
312.21 Phases of an investigation.
312.22 General principles of the IND submission.
312.23 IND content and format.
312.30 Protocol amendments.
312.31 Information amendments.
312.32 IND safety reports.
312.33 Annual reports.
312.34 Treatment use of an investigational new drug.
312.35 Submissions for treatment use.
312.36 Emergency use of an investigational new drug.
312.38 Withdrawal of an IND.

Subpart C—Administrative Actions

312.40 General requirements for use of an investigational new drug in a clinical investigation.
312.41 Comment and advice on an IND.
312.42 Clinical holds and requests for modification.
312.44 Termination.
312.45 Inactive status.
312.47 Meetings.
312.48 Dispute resolution.

Subpart D—Responsibilities of Sponsors and Investigators

312.50 General responsibilities of sponsors.
312.52 Transfer of obligations to a contract research organization.
312.53 Selecting investigators and monitors.
312.54 Emergency research under §50.24 of this chapter.
312.55 Informing investigators.
312.56 Review of ongoing investigations.
312.57 Recordkeeping and record retention.
312.58 Inspection of sponsor's records and reports.
312.59 Disposition of unused supply of investigational drug.
312.60 General responsibilities of investigators.
312.61 Control of the investigational drug.
312.62 Investigator recordkeeping and record retention.
312.64 Investigator reports.
312.66 Assurance of IRB review.
312.68 Inspection of investigator's records and reports.
312.69 Handling of controlled substances.
312.70 Disqualification of a clinical investigator.

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

312.80 Purpose.
312.81 Scope.
312.82 Early consultation.
312.83 Treatment protocols.
312.84 Risk-benefit analysis in review of marketing applications for drugs to treat life-threatening and severely-debilitating illnesses.
312.85 Phase 4 studies.
312.86 Focused FDA regulatory research.
312.87 Active monitoring of conduct and evaluation of clinical trials.
312.88 Safeguards for patient safety.

Subpart F—Miscellaneous

312.110 Import and export requirements.
312.120 Foreign clinical studies not conducted under an IND.
312.130 Availability for public disclosure of data and information in an IND.
312.140 Address for correspondence.
312.145 Guidelines.

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or in Vitro Tests

312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 371; 42 U.S.C. 262.

SOURCE: 52 FR 8831, Mar. 19, 1987, unless otherwise noted.

Subpart A—General Provisions

§ 312.1 Scope.

(a) This part contains procedures and requirements governing the use of investigational new drugs, including procedures and requirements for the submission to, and review by, the Food and Drug Administration of investigational new drug applications (IND's). An investigational new drug for which an IND is in effect in accordance with this part is exempt from the premarketing approval requirements that are otherwise applicable and may be shipped lawfully for the purpose of conducting clinical investigations of that drug.

(b) References in this part to regulations in the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

§ 312.2 Applicability.

(a) *Applicability.* Except as provided in this section, this part applies to all clinical investigations of products that are subject to section 505 or 507 of the Federal Food, Drug, and Cosmetic Act or to the licensing provisions of the Public Health Service Act (58 Stat. 632, as amended (42 U.S.C. 201 *et seq.*)).

(b) *Exemptions.* (1) The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of § 312.7.

(2)(i) A clinical investigation involving an in vitro diagnostic biological product listed in paragraph (b)(2)(ii) of this section is exempt from the requirements of this part if (*a*) it is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure and (*b*) it is shipped in compliance with § 312.160.

(ii) In accordance with paragraph (b)(2)(i) of this section, the following products are exempt from the requirements of this part: (*a*) blood grouping serum; (*b*) reagent red blood cells; and (*c*) anti-human globulin.

(3) A drug intended solely for tests in vitro or in laboratory research animals is exempt from the requirements of this part if shipped in accordance with § 312.160.

(4) FDA will not accept an application for an investigation that is exempt under the provisions of paragraph (b)(1) of this section.

(5) A clinical investigation involving use of a placebo is exempt from the requirements of this part if the investigation does not otherwise require submission of an IND.

(6) A clinical investigation involving an exception from informed consent under § 50.24 of this chapter is not exempt from the requirements of this part.

(c) *Bioavailability studies.* The applicability of this part to in vivo bioavailability studies in humans is subject to the provisions of § 320.31.

(d) *Unlabeled indication.* This part does not apply to the use in the practice of medicine for an unlabeled indication of a new drug or antibiotic drug product approved under part 314 or of a licensed biological product.

(e) *Guidance.* FDA may, on its own initiative, issue guidance on the applicability of this part to particular investigational uses of drugs. On request, FDA will advise on the applicability of this part to a planned clinical investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996]

§ 312.3 Definitions and interpretations.

(a) The definitions and interpretations of terms contained in section 201 of the Act apply to those terms when used in this part:

(b) The following definitions of terms also apply to this part:

Act means the Federal Food, Drug, and Cosmetic Act (secs. 201–902, 52 Stat. 1040 *et seq.*, as amended (21 U.S.C. 301–392)).

Clinical investigation means any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects. For the purposes of this part, an experiment is any use of a drug except for the use of a marketed drug in the course of medical practice.

Contract research organization means a person that assumes, as an independent contractor with the sponsor, one or more of the obligations of a sponsor, e.g., design of a protocol, selection or monitoring of investigations, evaluation of reports, and preparation of materials to be submitted to the Food and Drug Administration.

FDA means the Food and Drug Administration.

IND means an investigational new drug application. For purposes of this part, ''IND'' is synonymous with ''Notice of Claimed Investigational Exemption for a New Drug.''

Investigational new drug means a new drug, antibiotic drug, or biological drug that is used in a clinical investigation. The term also includes a biological product that is used in vitro for diagnostic purposes. The terms ''investigational drug'' and ''investigational new drug'' are deemed to be synonymous for purposes of this part.

Investigator means an individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the investigator is the responsible leader of the team. ''Subinvestigator'' includes any other individual member of that team.

Marketing application means an application for a new drug submitted under section 505(b) of the Act, a request to provide for certification of an antibiotic submitted under section 507 of the Act, or a product license application for a biological product submitted under the Public Health Service Act.

Sponsor means a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization. The sponsor does not actually conduct the investigation unless the sponsor is a sponsor-investigator. A person other than an individual that uses one or more of its own employees to conduct an investigation that it has initiated is a sponsor, not a sponsor-investigator, and the employees are investigators.

Sponsor-Investigator means an individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual. The requirements applicable to a sponsor-investigator under this part include both those applicable to an investigator and a sponsor.

Subject means a human who participates in an investigation, either as a recipient of the investigational new drug or as a control. A subject may be a healthy human or a patient with a disease.

§ 312.6 Labeling of an investigational new drug.

(a) The immediate package of an investigational new drug intended for human use shall bear a label with the

statement ''Caution: New Drug—Limited by Federal (or United States) law to investigational use.''

(b) The label or labeling of an investigational new drug shall not bear any statement that is false or misleading in any particular and shall not represent that the investigational new drug is safe or effective for the purposes for which it is being investigated.

§ 312.7 Promotion and charging for investigational drugs.

(a) *Promotion of an investigational new drug.* A sponsor or investigator, or any person acting on behalf of a sponsor or investigator, shall not represent in a promotional context that an investigational new drug is safe or effective for the purposes for which it is under investigation or otherwise promote the drug. This provision is not intended to restrict the full exchange of scientific information concerning the drug, including dissemination of scientific findings in scientific or lay media. Rather, its intent is to restrict promotional claims of safety or effectiveness of the drug for a use for which it is under investigation and to preclude commercialization of the drug before it is approved for commercial distribution.

(b) *Commercial distribution of an investigational new drug.* A sponsor or investigator shall not commercially distribute or test market an investigational new drug.

(c) *Prolonging an investigation.* A sponsor shall not unduly prolong an investigation after finding that the results of the investigation appear to establish sufficient data to support a marketing application.

(d) *Charging for and commercialization of investigational drugs*—(1) *Clinical trials under an IND.* Charging for an investigational drug in a clinical trial under an IND is not permitted without the prior written approval of FDA. In requesting such approval, the sponsor shall provide a full written explanation of why charging is necessary in order for the sponsor to undertake or continue the clinical trial, e.g., why distribution of the drug to test subjects should not be considered part of the normal cost of doing business.

(2) *Treatment protocol or treatment IND.* A sponsor or investigator may charge for an investigational drug for a treatment use under a treatment protocol or treatment IND provided: (i) There is adequate enrollment in the ongoing clinical investigations under the authorized IND; (ii) charging does not constitute commercial marketing of a new drug for which a marketing application has not been approved; (iii) the drug is not being commercially promoted or advertised; and (iv) the sponsor of the drug is actively pursuing marketing approval with due diligence. FDA must be notified in writing in advance of commencing any such charges, in an information amendment submitted under § 312.31. Authorization for charging goes into effect automatically 30 days after receipt by FDA of the information amendment, unless the sponsor is notified to the contrary.

(3) *Noncommercialization of investigational drug.* Under this section, the sponsor may not commercialize an investigational drug by charging a price larger than that necessary to recover costs of manufacture, research, development, and handling of the investigational drug.

(4) *Withdrawal of authorization.* Authorization to charge for an investigational drug under this section may be withdrawn by FDA if the agency finds that the conditions underlying the authorization are no longer satisfied.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19476, May 22, 1987]

§ 312.10 Waivers.

(a) A sponsor may request FDA to waive applicable requirement under this part. A waiver request may be submitted either in an IND or in an information amendment to an IND. In an emergency, a request may be made by telephone or other rapid communication means. A waiver request is required to contain at least one of the following:

(1) An explanation why the sponsor's compliance with the requirement is unnecessary or cannot be achieved;

(2) A description of an alternative submission or course of action that satisfies the purpose of the requirement; or

(3) Other information justifying a waiver.

(b) FDA may grant a waiver if it finds that the sponsor's noncompliance would not pose a significant and unreasonable risk to human subjects of the investigation and that one of the following is met:

(1) The sponsor's compliance with the requirement is unnecessary for the agency to evaluate the application, or compliance cannot be achieved;

(2) The sponsor's proposed alternative satisfies the requirement; or

(3) The applicant's submission otherwise justifies a waiver.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart B—Investigational New Drug Application (IND)

§ 312.20 Requirement for an IND.

(a) A sponsor shall submit an IND to FDA if the sponsor intends to conduct a clinical investigation with an investigational new drug that is subject to § 312.2(a).

(b) A sponsor shall not begin a clinical investigation subject to § 312.2(a) until the investigation is subject to an IND which is in effect in accordance with § 312.40.

(c) A sponsor shall submit a separate IND for any clinical investigation involving an exception from informed consent under § 50.24 of this chapter. Such a clinical investigation is not permitted to proceed without the prior written authorization from FDA. FDA shall provide a written determination 30 days after FDA receives the IND or earlier.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 62 FR 32479, June 16, 1997]

§ 312.21 Phases of an investigation.

An IND may be submitted for one or more phases of an investigation. The clinical investigation of a previously untested drug is generally divided into three phases. Although in general the phases are conducted sequentially, they may overlap. These three phases of an investigation are a follows:

(a) *Phase 1.* (1) Phase 1 includes the initial introduction of an investigational new drug into humans. Phase 1 studies are typically closely monitored and may be conducted in patients or normal volunteer subjects. These studies are designed to determine the metabolism and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies. The total number of subjects and patients included in Phase 1 studies varies with the drug, but is generally in the range of 20 to 80.

(2) Phase 1 studies also include studies of drug metabolism, structure-activity relationships, and mechanism of action in humans, as well as studies in which investigational drugs are used as research tools to explore biological phenomena or disease processes.

(b) *Phase 2.* Phase 2 includes the controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well controlled, closely monitored, and conducted in a relatively small number of patients, usually involving no more than several hundred subjects.

(c) *Phase 3.* Phase 3 studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug and to provide an adequate basis for physician labeling. Phase 3 studies usually include from several hundred to several thousand subjects.

§ 312.22 General principles of the IND submission.

(a) FDA's primary objectives in reviewing an IND are, in all phases of the investigation, to assure the safety and rights of subjects, and, in Phase 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. Therefore, although FDA's review of Phase 1 submissions will focus on assessing the safety of Phase 1 investigations, FDA's review of Phases 2 and 3 submissions will also include an assessment of the scientific quality of the clinical investigations and the likelihood that the investigations will yield data capable of meeting statutory standards for marketing approval.

(b) The amount of information on a particular drug that must be submitted in an IND to assure the accomplishment of the objectives described in paragraph (a) of this section depends upon such factors as the novelty of the drug, the extent to which it has been studied previously, the known or suspected risks, and the developmental phase of the drug.

(c) The central focus of the initial IND submission should be on the general investigational plan and the protocols for specific human studies. Subsequent amendments to the IND that contain new or revised protocols should build logically on previous submissions and should be supported by additional information, including the results of animal toxicology studies or other human studies as appropriate. Annual reports to the IND should serve as the focus for reporting the status of studies being conducted under the IND and should update the general investigational plan for the coming year.

(d) The IND format set forth in § 312.23 should be followed routinely by sponsors in the interest of fostering an efficient review of applications. Sponsors are expected to exercise considerable discretion, however, regarding the content of information submitted in each section, depending upon the kind of drug being studied and the nature of the available information. Section 312.23 outlines the information needed for a commercially sponsored IND for a new molecular entity. A sponsor-investigator who uses, as a research tool, an investigational new drug that is already subject to a manufacturer's IND or marketing application should follow the same general format, but ordinarily may, if authorized by the manufacturer, refer to the manufacturer's IND or marketing application in providing the technical information supporting the proposed clinical investigation. A sponsor-investigator who uses an investigational drug not subject to a manufacturer's IND or marketing application is ordinarily required to submit all technical information supporting the IND, unless such information may be referenced from the scientific literature.

§ 312.23 IND content and format.

(a) A sponsor who intends to conduct a clinical investigation subject to this part shall submit an ''Investigational New Drug Application'' (IND) including, in the following order:

(1) *Cover sheet (Form FDA–1571).* A cover sheet for the application containing the following:

(i) The name, address, and telephone number of the sponsor, the date of the application, and the name of the investigational new drug.

(ii) Identification of the phase or phases of the clinical investigation to be conducted.

(iii) A commitment not to begin clinical investigations until an IND covering the investigations is in effect.

(iv) A commitment that an Institutional Review Board (IRB) that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of each of the studies in the proposed clinical investigation and that the investigator will report to the IRB proposed changes in the research activity in accordance with the requirements of part 56.

(v) A commitment to conduct the investigation in accordance with all other applicable regulatory requirements.

(vi) The name and title of the person responsible for monitoring the conduct and progress of the clinical investigations.

(vii) The name(s) and title(s) of the person(s) responsible under § 312.32 for

review and evaluation of information relevant to the safety of the drug.

(viii) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer—in lieu of a listing of the specific obligations transferred—may be submitted.

(ix) The signature of the sponsor or the sponsor's authorized representative. If the person signing the application does not reside or have a place of business within the United States, the IND is required to contain the name and address of, and be countersigned by, an attorney, agent, or other authorized official who resides or maintains a place of business within the United States.

(2) *A table of contents.*

(3) *Introductory statement and general investigational plan.* (i) A brief introductory statement giving the name of the drug and all active ingredients, the drug's pharmacological class, the structural formula of the drug (if known), the formulation of the dosage form(s) to be used, the route of administration, and the broad objectives and planned duration of the proposed clinical investigation(s).

(ii) A brief summary of previous human experience with the drug, with reference to other IND's if pertinent, and to investigational or marketing experience in other countries that may be relevant to the safety of the proposed clinical investigation(s).

(iii) If the drug has been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness, identification of the country(ies) where the drug was withdrawn and the reasons for the withdrawal.

(iv) A brief description of the overall plan for investigating the drug product for the following year. The plan should include the following: (*a*) The rationale for the drug or the research study; (*b*) the indication(s) to be studied; (*c*) the general approach to be followed in evaluating the drug; (*d*) the kinds of clinical trials to be conducted in the first year following the submission (if plans are not developed for the entire year, the sponsor should so indicate); (*e*) the estimated number of patients to be given the drug in those studies; and (*f*) any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs.

(4) [Reserved]

(5) *Investigator's brochure.* If required under § 312.55, a copy of the investigator's brochure, containing the following information:

(i) A brief description of the drug substance and the formulation, including the structural formula, if known.

(ii) A summary of the pharmacological and toxicological effects of the drug in animals and, to the extent known, in humans.

(iii) A summary of the pharmacokinetics and biological disposition of the drug in animals and, if known, in humans.

(iv) A summary of information relating to safety and effectiveness in humans obtained from prior clinical studies. (Reprints of published articles on such studies may be appended when useful.)

(v) A description of possible risks and side effects to be anticipated on the basis of prior experience with the drug under investigation or with related drugs, and of precautions or special monitoring to be done as part of the investigational use of the drug.

(6) *Protocols.* (i) A protocol for each planned study. (Protocols for studies not submitted initially in the IND should be submitted in accordance with § 312.30(a).) In general, protocols for Phase 1 studies may be less detailed and more flexible than protocols for Phase 2 and 3 studies. Phase 1 protocols should be directed primarily at providing an outline of the investigation—an estimate of the number of patients to be involved, a description of safety exclusions, and a description of the dosing plan including duration, dose, or method to be used in determining dose—and should specify in detail only those elements of the study that are critical to safety, such as necessary

monitoring of vital signs and blood chemistries. Modifications of the experimental design of Phase 1 studies that do not affect critical safety assessments are required to be reported to FDA only in the annual report.

(ii) In Phases 2 and 3, detailed protocols describing all aspects of the study should be submitted. A protocol for a Phase 2 or 3 investigation should be designed in such a way that, if the sponsor anticipates that some deviation from the study design may become necessary as the investigation progresses, alternatives or contingencies to provide for such deviation are built into the protocols at the outset. For example, a protocol for a controlled short-term study might include a plan for an early crossover of nonresponders to an alternative therapy.

(iii) A protocol is required to contain the following, with the specific elements and detail of the protocol reflecting the above distinctions depending on the phase of study:

(*a*) A statement of the objectives and purpose of the study.

(*b*) The name and address and a statement of the qualifications (curriculum vitae or other statement of qualifications) of each investigator, and the name of each subinvestigator (e.g., research fellow, resident) working under the supervision of the investigator; the name and address of the research facilities to be used; and the name and address of each reviewing Institutional Review Board.

(*c*) The criteria for patient selection and for exclusion of patients and an estimate of the number of patients to be studied.

(*d*) A description of the design of the study, including the kind of control group to be used, if any, and a description of methods to be used to minimize bias on the part of subjects, investigators, and analysts.

(*e*) The method for determining the dose(s) to be administered, the planned maximum dosage, and the duration of individual patient exposure to the drug.

(*f*) A description of the observations and measurements to be made to fulfill the objectives of the study.

(*g*) A description of clinical procedures, laboratory tests, or other measures to be taken to monitor the effects of the drug in human subjects and to minimize risk.

(7) *Chemistry, manufacturing, and control information.* (i) As appropriate for the particular investigations covered by the IND, a section describing the composition, manufacture, and control of the drug substance and the drug product. Although in each phase of the investigation sufficient information is required to be submitted to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available. FDA recognizes that modifications to the method of preparation of the new drug substance and dosage form and changes in the dosage form itself are likely as the investigation progresses. Therefore, the emphasis in an initial Phase 1 submission should generally be placed on the identification and control of the raw materials and the new drug substance. Final specifications for the drug substance and drug product are not expected until the end of the investigational process.

(ii) It should be emphasized that the amount of information to be submitted depends upon the scope of the proposed clinical investigation. For example, although stability data are required in all phases of the IND to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation, if very short-term tests are proposed, the supporting stability data can be correspondingly limited.

(iii) As drug development proceeds and as the scale or production is changed from the pilot-scale production appropriate for the limited initial clinical investigations to the larger-scale production needed for expanded clinical trials, the sponsor should submit information amendments to supplement the initial information submitted on the chemistry, manufacturing, and control processes with information appropriate to the expanded scope of the investigation.

(iv) Reflecting the distinctions described in this paragraph (a)(7), and based on the phase(s) to be studied, the submission is required to contain the following:

(*a*) *Drug substance.* A description of the drug substance, including its physical, chemical, or biological characteristics; the name and address of its manufacturer; the general method of preparation of the drug substance; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance; and information sufficient to support stability of the drug substance during the toxicological studies and the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy relevant requirements in this paragraph.

(*b*) *Drug product.* A list of all components, which may include reasonable alternatives for inactive compounds, used in the manufacture of the investigational drug product, including both those components intended to appear in the drug product and those which may not appear but which are used in the manufacturing process, and, where applicable, the quantitative composition of the investigational drug product, including any reasonable variations that may be expected during the investigational stage; the name and address of the drug product manufacturer; a brief general description of the manufacturing and packaging procedure as appropriate for the product; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug product; and information sufficient to assure the product's stability during the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy certain requirements in this paragraph.

(*c*) A brief general description of the composition, manufacture, and control of any placebo used in a controlled clinical trial.

(*d*) *Labeling.* A copy of all labels and labeling to be provided to each investigator.

(E) *Environmental analysis requirements.* A claim for categorical exclusion under § 25.30 or 25.31 or an environmental assessment under § 25.40.

(8) *Pharmacology and toxicology information.* Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations. Guidelines are available from FDA that describe ways in which these requirements may be met. Such information is required to include the identification and qualifications of the individuals who evaluated the results of such studies and concluded that it is reasonably safe to begin the proposed investigations and a statement of where the investigations were conducted and where the records are available for inspection. As drug development proceeds, the sponsor is required to submit informational amendments, as appropriate, with additional information pertinent to safety.

(i) *Pharmacology and drug disposition.* A section describing the pharmacological effects and mechanism(s) of action of the drug in animals, and information on the absorption, distribution, metabolism, and excretion of the drug, if known.

(ii) *Toxicology.* (*a*) An integrated summary of the toxicological effects of the drug in animals and in vitro. Depending on the nature of the drug and the phase of the investigation, the description is to include the results of acute, subacute, and chronic toxicity tests; tests of the drug's effects on reproduction and the developing fetus; any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology); and any in vitro studies intended to evaluate drug toxicity.

(*b*) For each toxicology study that is intended primarily to support the safety of the proposed clinical investigation, a full tabulation of data suitable for detailed review.

(iii) For each nonclinical laboratory study subject to the good laboratory practice regulations under part 58, a

statement that the study was conducted in compliance with the good laboratory practice regulations in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance.

(9) *Previous human experience with the investigational drug.* A summary of previous human experience known to the applicant, if any, with the investigational drug. The information is required to include the following:

(i) If the investigational drug has been investigated or marketed previously, either in the United States or other countries, detailed information about such experience that is relevant to the safety of the proposed investigation or to the investigation's rationale. If the durg has been the subject of controlled trials, detailed information on such trials that is relevant to an assessment of the drug's effectiveness for the proposed investigational use(s) should also be provided. Any published material that is relevant to the safety of the proposed investigation or to an assessment of the drug's effectiveness for its proposed investigational use should be provided in full. Published material that is less directly relevant may be supplied by a bibliography.

(ii) If the drug is a combination of drugs previously investigated or marketed, the information required under paragraph (a)(9)(i) of this section should be provided for each active drug component. However, if any component in such combination is subject to an approved marketing application or is otherwise lawfully marketed in the United States, the sponsor is not required to submit published material concerning that active drug component unless such material relates directly to the proposed investigational use (including publications relevant to component-component interaction).

(iii) If the drug has been marketed outside the United States, a list of the countries in which the drug has been marketed and a list of the countries in which the drug has been withdrawn from marketing for reasons potentially related to safety or effectiveness.

(10) *Additional information.* In certain applications, as described below, information on special topics may be needed. Such information shall be submitted in this section as follows:

(i) *Drug dependence and abuse potential.* If the drug is a psychotropic substance or otherwise has abuse potential, a section describing relevant clinical studies and experience and studies in test animals.

(ii) *Radioactive drugs.* If the drug is a radioactive drug, sufficient data from animal or human studies to allow a reasonable calculation of radiation-absorbed dose to the whole body and critical organs upon administration to a human subject. Phase 1 studies of radioactive drugs must include studies which will obtain sufficient data for dosimetry calculations.

(iii) *Other information.* A brief statement of any other information that would aid evaluation of the proposed clinical investigations with respect to their safety or their design and potential as controlled clinical trials to support marketing of the drug.

(11) *Relevant information.* If requested by FDA, any other relevant information needed for review of the application.

(b) *Information previously submitted.* The sponsor ordinarily is not required to resubmit information previously submitted, but may incorporate the information by reference. A reference to information submitted previously must identify the file by name, reference number, volume, and page number where the information can be found. A reference to information submitted to the agency by a person other than the sponsor is required to contain a written statement that authorizes the reference and that is signed by the person who submitted the information.

(c) *Material in a foreign language.* The sponsor shall submit an accurate and complete English translation of each part of the IND that is not in English. The sponsor shall also submit a copy of each original literature publication for which an English translation is submitted.

(d) *Number of copies.* The sponsor shall submit an original and two copies of all submissions to the IND file, including the original submission and all amendments and reports.

(e) *Numbering of IND submissions.* Each submission relating to an IND is

required to be numbered serially using a single, three-digit serial number. The initial IND is required to be numbered 000; each subsequent submission (e.g., amendment, report, or correspondence) is required to be numbered chronologically in sequence.

(f) *Identification of exception from informed consent.* If the investigation involves an exception from informed consent under § 50.24 of this chapter, the sponsor shall prominently identify on the cover sheet that the investigation is subject to the requirements in § 50.24 of this chapter.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51529, Oct. 2, 1996; 62 FR 40599, July 29, 1997]

§ 312.30 Protocol amendments.

Once an IND is in effect, a sponsor shall amend it as needed to ensure that the clinical investigations are conducted according to protocols included in the application. This section sets forth the provisions under which new protocols may be submitted and changes in previously submitted protocols may be made. Whenever a sponsor intends to conduct a clinical investigation with an exception from informed consent for emergency research as set forth in § 50.24 of this chapter, the sponsor shall submit a separate IND for such investigation.

(a) *New protocol.* Whenever a sponsor intends to conduct a study that is not covered by a protocol already contained in the IND, the sponsor shall submit to FDA a protocol amendment containing the protocol for the study. Such study may begin provided two conditions are met: (1) The sponsor has submitted the protocol to FDA for its review; and (2) the protocol has been approved by the Institutional Review Board (IRB) with responsibility for review and approval of the study in accordance with the requirements of part 56. The sponsor may comply with these two conditions in either order.

(b) *Changes in a protocol.* (1) A sponsor shall submit a protocol amendment describing any change in a Phase 1 protocol that significantly affects the safety of subjects or any change in a Phase 2 or 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of changes requiring an amendment under this paragraph include:

(i) Any increase in drug dosage or duration of exposure of individual subjects to the drug beyond that in the current protocol, or any significant increase in the number of subjects under study.

(ii) Any significant change in the design of a protocol (such as the addition or dropping of a control group).

(iii) The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor safety.

(2)(i) A protocol change under paragraph (b)(1) of this section may be made provided two conditions are met:

(*a*) The sponsor has submitted the change to FDA for its review; and

(*b*) The change has been approved by the IRB with responsibility for review and approval of the study. The sponsor may comply with these two conditions in either order.

(ii) Notwithstanding paragraph (b)(2)(i) of this section, a protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately provided FDA is subsequently notified by protocol amendment and the reviewing IRB is notified in accordance with § 56.104(c).

(c) *New investigator.* A sponsor shall submit a protocol amendment when a new investigator is added to carry out a previously submitted protocol, except that a protocol amendment is not required when a licensed practitioner is added in the case of a treatment protocol under § 312.34. Once the investigator is added to the study, the investigational drug may be shipped to the investigator and the investigator may begin participating in the study. The sponsor shall notify FDA of the new investigator within 30 days of the investigator being added.

(d) *Content and format.* A protocol amendment is required to be prominently identified as such (i.e., ''Protocol Amendment: New Protocol'', ''Protocol Amendment: Change in Protocol'', or ''Protocol Amendment: New Investigator''), and to contain the following:

(1)(i) In the case of a new protocol, a copy of the new protocol and a brief description of the most clinically significant differences between it and previous protocols.

(ii) In the case of a change in protocol, a brief description of the change and reference (date and number) to the submission that contained the protocol.

(iii) In the case of a new investigator, the investigator's name, the qualifications to conduct the investigation, reference to the previously submitted protocol, and all additional information about the investigator's study as is required under § 312.23(a)(6)(iii)(*b*).

(2) Reference, if necessary, to specific technical information in the IND or in a concurrently submitted information amendment to the IND that the sponsor relies on to support any clinically significant change in the new or amended protocol. If the reference is made to supporting information already in the IND, the sponsor shall identify by name, reference number, volume, and page number the location of the information.

(3) If the sponsor desires FDA to comment on the submission, a request for such comment and the specific questions FDA's response should address.

(e) *When submitted.* A sponsor shall submit a protocol amendment for a new protocol or a change in protocol before its implementation. Protocol amendments to add a new investigator or to provide additional information about investigators may be grouped and submitted at 30-day intervals. When several submissions of new protocols or protocol changes are anticipated during a short period, the sponsor is encouraged, to the extent feasible, to include these all in a single submission.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51530, Oct. 2, 1996]

§ 312.31 Information amendments.

(a) *Requirement for information amendment.* A sponsor shall report in an information amendment essential information on the IND that is not within the scope of a protocol amendment, IND safety reports, or annual report. Examples of information requiring an information amendment include:

(1) New toxicology, chemistry, or other technical information; or

(2) A report regarding the discontinuance of a clinical investigation.

(b) *Content and format of an information amendment.* An information amendment is required to bear prominent identification of its contents (e.g., ''Information Amendment: Chemistry, Manufacturing, and Control'', ''Information Amendment: Pharmacology-Toxicology'', ''Information Amendment: Clinical''), and to contain the following:

(1) A statement of the nature and purpose of the amendment.

(2) An organized submission of the data in a format appropriate for scientific review.

(3) If the sponsor desires FDA to comment on an information amendment, a request for such comment.

(c) *When submitted.* Information amendments to the IND should be submitted as necessary but, to the extent feasible, not more than every 30 days.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988]

§ 312.32 IND safety reports.

(a) *Definitions.* The following definitions of terms apply to this section:–

Associated with the use of the drug. There is a reasonable possibility that the experience may have been caused by the drug.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. ''Unexpected,'' as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) *Review of safety information.* The sponsor shall promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the agency by the sponsor.

(c) *IND safety reports.* (1) *Written reports*—(i) The sponsor shall notify FDA and all participating investigators in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., ''IND Safety Report.'' Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility

for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previouos, similar reports.

(2) *Telephone and facsimile transmission safety reports.* The sponsor shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than 7 calendar days after the sponsor's initial receipt of the information. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the new drug review division in the Center for Drug Evaluation and Research or the director of the products review division in the Center for Biologics Evaluation and Research which is responsible for review of the IND.

(4) A sponsor of a clinical study of a marketed drug is not required to make a safety report for any adverse experience associated with use of the drug that is not from the clinical study itself.

(d) *Followup.* (1) The sponsor shall promptly investigate all safety information received by it.

(2) Followup information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable under paragraph (c) of this section is so reportable, the sponsor shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of a sponsor's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report.

(e) *Disclaimer.* A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse experience. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse experience.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 62 FR 52250, Oct. 7, 1997]

EFFECTIVE DATE NOTE: At 62 FR 52250, Oct. 7, 1997, § 312.32 was amended by revising paragraphs (a), (b), (c)(1)(i), (c)(2), and (d)(3); by adding in the second sentence of paragraph (c)(3) the words ''new drug review'' before the phrase ''division in the Center for Drug Evaluation and Research'' and the words ''the director of the product review division in'' before the phrase ''the Center for Biologics Evaluation and Research''; and by removing in paragraph (e) the word ''section'' and replacing it with the word ''part'', effective Apr. 6, 1998. For the convenience of the user, the superseded text is set forth as follows:

§ 312.32 IND safety reports.

(a) *Definitions.* The following definitions of terms apply to this section:

Associated with the use of the drug means that there is a reasonable possibility that the experience may have been caused by the drug.

Serious adverse experience means any experience that suggests a significant hazard, contraindication, side effect, or precaution. With respect to human clinical experience, a serious adverse drug experience includes any experience that is fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose. With respect to results obtained from tests in laboratory animals, a

serious adverse drug experience includes any experience suggesting a significant risk for human subjects, including any finding of mutagenicity, teratogenicity, or carcinogenicity.

Unexpected adverse experience means any adverse experience that is not identified in nature, severity, or frequency in the current investigator brochure; or, if an investigator brochure is not required, that is not identified in nature, severity, or freuquency in the risk information described in the general investigational plan or elsewhere in the current application, as amended.

(b) *Review of safety information.* The sponsor shall promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from any source, foreign or domestic, including information derived from clinical investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers.

(c) *IND safety reports.* (1) *Written reports.* (i) The sponsor shall notify FDA and all participating investigators in a written IND safety report of any adverse experience associated with use of the drug that is both serious and unexpected. Such notification shall be made as soon as possible and in no event later than 10 working days after the sponsor's initial receipt of the information. Each written notification shall bear prominent identification of its contents, i.e., ''IND Safety Report.'' Each written notification to FDA shall be transmitted to the FDA division of the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which has responsibility for review of the IND.

(ii)* * *

(2) *Telephone report.* The sponsor shall also notify FDA by telephone of any unexpected fatal or life-threatening experience associated with use of the drug in the clinical studies conducted under the IND no later than 3 working days after receipt of the information. Each telephone call to FDA shall be transmitted to the FDA division of the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which has responsibility for review of the IND. For purposes of this section, life-threatening means that the patient was, in the view of the investigator, at *immediate* (emphasis added) risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

* * * * *

(d)* * *

(3) If the results of a sponsor's investigation show that an adverse experience not initially determined to be reportable under paragraph (c) of this section is so reportable, the sponsor shall report such experience in a safety report as soon as possible after the determination is made, but in no event longer than 10-working days.

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§ 312.33 Annual reports.

A sponsor shall within 60 days of the anniversary date that the IND went into effect, submit a brief report of the progress of the investigation that includes:

(a) *Individual study information.* A brief summary of the status of each study in progress and each study completed during the previous year. The summary is required to include the following information for each study:

(1) The title of the study (with any appropriate study identifiers such as protocol number), its purpose, a brief statement identifying the patient population, and a statement as to whether the study is completed.

(2) The total number of subjects initially planned for inclusion in the study; the number entered into the study to date, tabulated by age group, gender, and race; the number whose participation in the study was completed as planned; and the number who dropped out of the study for any reason.

(3) If the study has been completed, or if interim results are known, a brief description of any available study results.

(b) *Summary information.* Information obtained during the previous year's clinical and nonclinical investigations, including:

(1) A narrative or tabular summary showing the most frequent and most serious adverse experiences by body system.

(2) A summary of all IND safety reports submitted during the past year.

(3) A list of subjects who died during participation in the investigation, with the cause of death for each subject.

(4) A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related.

(5) A brief description of what, if anything, was obtained that is pertinent to an understanding of the drug's actions, including, for example, information about dose response, information from controlled trails, and information about bioavailability.

(6) A list of the preclinical studies (including animal studies) completed or in progress during the past year and a summary of the major preclinical findings.

(7) A summary of any significant manufacturing or microbiological changes made during the past year.

(c) A description of the general investigational plan for the coming year to replace that submitted 1 year earlier. The general investigational plan shall contain the information required under § 312.23(a)(3)(iv).

(d) If the investigator brochure has been revised, a description of the revision and a copy of the new brochure.

(e) A description of any significant Phase 1 protocol modifications made during the previous year and not previously reported to the IND in a protocol amendment.

(f) A brief summary of significant foreign marketing developments with the drug during the past year, such as approval of marketing in any country or withdrawal or suspension from marketing in any country.

(g) If desired by the sponsor, a log of any outstanding business with respect to the IND for which the sponsor requests or expects a reply, comment, or meeting.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 6862, Feb. 11, 1998]

EFFECTIVE DATE NOTE: At 63 FR 6862, Feb. 11, 1998, § 312.33 was amended by revising paragraph (a)(2), effective Aug. 10, 1998. For the convenience of the user, the superseded text is set forth as follows:

§ 312.33 Annual reports.

* * * * *

(a) * * *

(2) The total number of subjects initially planned for inclusion in the study, the number entered into the study to date, the number whose participation in the study was completed as planned, and the number who dropped out of the study for any reason.

* * * * *

§ 312.34 Treatment use of an investigational new drug.

(a) *General.* A drug that is not approved for marketing may be under clinical investigation for a serious or immediately life-threatening disease condition in patients for whom no comparable or satisfactory alternative drug or other therapy is available. During the clinical investigation of the drug, it may be appropriate to use the drug in the treatment of patients not in the clinical trials, in accordance with a treatment protocol or treatment IND. The purpose of this section is to facilitate the availability of promising new drugs to desperately ill patients as early in the drug development process as possible, before general marketing begins, and to obtain additional data on the drug's safety and effectiveness. In the case of a serious disease, a drug ordinarily may be made available for treatment use under this section during Phase 3 investigations or after all clinical trials have been completed; however, in appropriate circumstances, a drug may be made available for treatment use during Phase 2. In the case of an immediately life-threatening disease, a drug may be made available for treatment use under this section earlier than Phase 3, but ordinarily not earlier than Phase 2. For purposes of this section, the ''treatment use'' of a drug includes the use of a drug for diagnostic purposes. If a protocol for an investigational drug meets the criteria of this section, the protocol is to be submitted as a treatment protocol under the provisions of this section.

(b) *Criteria.* (1) FDA shall permit an investigational drug to be used for a treatment use under a treatment protocol or treatment IND if:

(i) The drug is intended to treat a serious or immediately life-threatening disease;

(ii) There is no comparable or satisfactory alternative drug or other therapy available to treat that stage of the

disease in the intended patient population;

(iii) The drug is under investigation in a controlled clinical trial under an IND in effect for the trial, or all clinical trials have been completed; and

(iv) The sponsor of the controlled clinical trial is actively pursuing marketing approval of the investigational drug with due diligence.

(2) *Serious disease.* For a drug intended to treat a serious disease, the Commissioner may deny a request for treatment use under a treatment protocol or treatment IND if there is insufficient evidence of safety and effectiveness to support such use.

(3) *Immediately life-threatening disease.* (i) For a drug intended to treat an immediately life-threatening disease, the Commissioner may deny a request for treatment use of an investigational drug under a treatment protocol or treatment IND if the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(A) May be effective for its intended use in its intended patient population; or

(B) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(ii) For the purpose of this section, an ''immediately life-threatening'' disease means a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment.

(c) *Safeguards.* Treatment use of an investigational drug is conditioned on the sponsor and investigators complying with the safeguards of the IND process, including the regulations governing informed consent (21 CFR part 50) and institutional review boards (21 CFR part 56) and the applicable provisions of part 312, including distribution of the drug through qualified experts, maintenance of adequate manufacturing facilities, and submission of IND safety reports.

(d) *Clinical hold.* FDA may place on clinical hold a proposed or ongoing treatment protocol or treatment IND in accordance with § 312.42.

[52 FR 19476, May 22, 1987, as amended at 57 FR 13248, Apr. 15, 1992]

§ 312.35 Submissions for treatment use.

(a) *Treatment protocol submitted by IND sponsor.* Any sponsor of a clinical investigation of a drug who intends to sponsor a treatment use for the drug shall submit to FDA a treatment protocol under § 312.34 if the sponsor believes the criteria of § 312.34 are satisfied. If a protocol is not submitted under § 312.34, but FDA believes that the protocol should have been submitted under this section, FDA may deem the protocol to be submitted under § 312.34. A treatment use under a treatment protocol may begin 30 days after FDA receives the protocol or on earlier notification by FDA that the treatment use described in the protocol may begin.

(1) A treatment protocol is required to contain the following:

(i) The intended use of the drug.

(ii) An explanation of the rationale for use of the drug, including, as appropriate, either a list of what available regimens ordinarily should be tried before using the investigational drug or an explanation of why the use of the investigational drug is preferable to the use of available marketed treatments.

(iii) A brief description of the criteria for patient selection.

(iv) The method of administration of the drug and the dosages.

(v) A description of clinical procedures, laboratory tests, or other measures to monitor the effects of the drug and to minimize risk.

(2) A treatment protocol is to be supported by the following:

(i) Informational brochure for supplying to each treating physician.

(ii) The technical information that is relevant to safety and effectiveness of the drug for the intended treatment purpose. Information contained in the sponsor's IND may be incorporated by reference.

(iii) A commitment by the sponsor to assure compliance of all participating investigators with the informed consent requirements of 21 CFR part 50.

(3) A licensed practioner who receives an investigational drug for treatment use under a treatment protocol is an ''investigator'' under the protocol and is responsible for meeting all applicable investigator responsibilities under this part and 21 CFR parts 50 and 56.

(b) *Treatment IND submitted by licensed practitioner.* (1) If a licensed medical practitioner wants to obtain an investigational drug subject to a controlled clinical trial for a treatment use, the practitioner should first attempt to obtain the drug from the sponsor of the controlled trial under a treatment protocol. If the sponsor of the controlled clinical investigation of the drug will not establish a treatment protocol for the drug under paragraph (a) of this section, the licensed medical practitioner may seek to obtain the drug from the sponsor and submit a treatment IND to FDA requesting authorization to use the investigational drug for treatment use. A treatment use under a treatment IND may begin 30 days after FDA receives the IND or on earlier notification by FDA that the treatment use under the IND may begin. A treatment IND is required to contain the following:

(i) A cover sheet (Form FDA 1571) meeting § 312.23(g)(1).

(ii) Information (when not provided by the sponsor) on the drug's chemistry, manufacturing, and controls, and prior clinical and nonclinical experience with the drug submitted in accordance with § 312.23. A sponsor of a clinical investigation subject to an IND who supplies an investigational drug to a licensed medical practitioner for purposes of a separate treatment clinical investigation shall be deemed to authorize the incorporation-by-reference of the technical information contained in the sponsor's IND into the medical practitioner's treatment IND.

(iii) A statement of the steps taken by the practitioner to obtain the drug under a treatment protocol from the drug sponsor.

(iv) A treatment protocol containing the same information listed in paragraph (a)(1) of this section.

(v) A statement of the practitioner's qualifications to use the investigational drug for the intended treatment use.

(vi) The practitioner's statement of familiarity with information on the drug's safety and effectiveness derived from previous clinical and nonclinical experience with the drug.

(vii) Agreement to report to FDA safety information in accordance with § 312.32.

(2) A licensed practitioner who submits a treatment IND under this section is the sponsor-investigator for such IND and is responsible for meeting all applicable sponsor and investigator responsibilities under this part and 21 CFR parts 50 and 56.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 19477, May 22, 1987, as amended at 57 FR 13249, Apr. 15, 1992]

§ 312.36 Emergency use of an investigational new drug.

Need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND in accordance with § 312.23 or § 312.34. In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND. A request for such authorization may be transmitted to FDA by telephone or other rapid communication means. For investigational biological drugs, the request should be directed to the Division of Biological Investigational New Drugs (HFB–230), Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892, 301–443–4864. For all other investigational drugs, the request for authorization should be directed to the Document Management and Reporting Branch (HFD–53), Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857, 301–443–4320. After normal working hours, eastern standard time, the request should be directed to the FDA Division of Emergency and Epidemiological Operations, 202–857–8400. Except in extraordinary circumstances, such authorization will be conditioned on the sponsor making an appropriate IND submission as soon

as practicable after receiving the authorization.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990]

§ 312.38 Withdrawal of an IND.

(a) At any time a sponsor may withdraw an effective IND without prejudice.

(b) If an IND is withdrawn, FDA shall be so notified, all clinical investigations conducted under the IND shall be ended, all current investigators notified, and all stocks of the drug returned to the sponsor or otherwise disposed of at the request of the sponsor in accordance with § 312.59.

(c) If an IND is withdrawn because of a safety reason, the sponsor shall promptly so inform FDA, all participating investigators, and all reviewing Institutional Review Boards, together with the reasons for such withdrawal.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart C—Administrative Actions

§ 312.40 General requirements for use of an investigational new drug in a clinical investigation.

(a) An investigational new drug may be used in a clinical investigation if the following conditions are met:

(1) The sponsor of the investigation submits an IND for the drug to FDA; the IND is in effect under paragraph (b) of this section; and the sponsor complies with all applicable requirements in this part and parts 50 and 56 with respect to the conduct of the clinical investigations; and

(2) Each participating investigator conducts his or her investigation in compliance with the requirements of this part and parts 50 and 56.

(b) An IND goes into effect:

(1) Thirty days after FDA receives the IND, unless FDA notifies the sponsor that the investigations described in the IND are subject to a clinical hold under § 312.42; or

(2) On earlier notification by FDA that the clinical investigations in the IND may begin. FDA will notify the sponsor in writing of the date it receives the IND.

(c) A sponsor may ship an investigational new drug to investigators named in the IND:

(1) Thirty days after FDA receives the IND; or

(2) On earlier FDA authorization to ship the drug.

(d) An investigator may not administer an investigational new drug to human subjects until the IND goes into effect under paragraph (b) of this section.

§ 312.41 Comment and advice on an IND.

(a) FDA may at any time during the course of the investigation communicate with the sponsor orally or in writing about deficiencies in the IND or about FDA's need for more data or information.

(b) On the sponsor's request, FDA will provide advice on specific matters relating to an IND. Examples of such advice may include advice on the adequacy of technical data to support an investigational plan, on the design of a clinical trial, and on whether proposed investigations are likely to produce the data and information that is needed to meet requirements for a marketing application.

(c) Unless the communication is accompanied by a clinical hold order under § 312.42, FDA communications with a sponsor under this section are solely advisory and do not require any modification in the planned or ongoing clinical investigations or response to the agency.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.42 Clinical holds and requests for modification.

(a) *General.* A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to

suspend an ongoing investigation. The clinical hold order may apply to one or more of the investigations covered by an IND. When a proposed study is placed on clinical hold, subjects may not be given the investigational drug. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by FDA in the interest of patient safety.

(b) *Grounds for imposition of clinical hold*—(1) *Clinical hold of a Phase 1 study under an IND.* FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that:

(i) Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury;

(ii) The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND;

(iii) The investigator brochure is misleading, erroneous, or materially incomplete; or

(iv) The IND does not contain sufficient information required under § 312.23 to assess the risks to subjects of the proposed studies.

(2) *Clinical hold of a Phase 2 or 3 study under an IND.* FDA may place a proposed or ongoing Phase 2 or 3 investigation on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1)(i) through (iv) of this section apply; or

(ii) The plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

(3) Clinical hold of a treatment IND or treatment protocol.

(i) *Proposed use.* FDA may place a proposed treatment IND or treatment protocol on clinical hold if it is determined that:

(A) The pertinent criteria in § 312.34(b) for permitting the treatment use to begin are not satisfied; or

(B) The treatment protocol or treatment IND does not contain the information required under § 312.35 (a) or (b) to make the specified determination under § 312.34(b).

(ii) *Ongoing use.* FDA may place an ongoing treatment protocol or treatment IND on clinical hold if it is determined that:

(A) There becomes available a comparable or satisfactory alternative drug or other therapy to treat that stage of the disease in the intended patient population for which the investigational drug is being used;

(B) The investigational drug is not under investigation in a controlled clinical trial under an IND in effect for the trial and not all controlled clinical trials necessary to support a marketing application have been completed, or a clinical study under the IND has been placed on clinical hold:

(C) The sponsor of the controlled clinical trial is not pursuing marketing approval with due diligence;

(D) If the treatment IND or treatment protocol is intended for a serious disease, there is insufficient evidence of safety and effectiveness to support such use; or

(E) If the treatment protocol or treatment IND was based on an immediately life-threatening disease, the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(*1*) May be effective for its intended use in its intended population; or

(*2*) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(iii) FDA may place a proposed or ongoing treatment IND or treatment protocol on clinical hold if it finds that any of the conditions in paragraph (b)(4)(i) through (b)(4)(viii) of this section apply.

(4) *Clinical hold of any study that is not designed to be adequate and well-controlled.* FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1) or (b)(2) of this section apply; or

(ii) There is reasonable evidence the investigation that is not designed to be adequate and well-controlled is impeding enrollment in, or otherwise interfering with the conduct or completion of, a study that is designed to be an

adequate and well-controlled investigation of the same or another investigational drug; or

(iii) Insufficient quantities of the investigational drug exist to adequately conduct both the investigation that is not designed to be adequate and well-controlled and the investigations that are designed to be adequate and well-controlled; or

(iv) The drug has been studied in one or more adequate and well-controlled investigations that strongly suggest lack of effectiveness; or

(v) Another drug under investigation or approved for the same indication and available to the same patient population has demonstrated a better potential benefit/risk balance; or

(vi) The drug has received marketing approval for the same indication in the same patient population; or

(vii) The sponsor of the study that is designed to be an adequate and well-controlled investigation is not actively pursuing marketing approval of the investigational drug with due diligence; or

(viii) The Commissioner determines that it would not be in the public interest for the study to be conducted or continued. FDA ordinarily intends that clinical holds under paragraphs (b)(4)(ii), (b)(4)(iii) and (b)(4)(v) of this section would only apply to additional enrollment in nonconcurrently controlled trials rather than eliminating continued access to individuals already receiving the investigational drug.

(5) *Clinical hold of any investigation involving an exception from informed consent under §50.24 of this chapter.* FDA may place a proposed or ongoing investigation involving an exception from informed consent under §50.24 of this chapter on clinical hold if it is determined that:

(i) Any of the conditions in paragraphs (b)(1) or (b)(2) of this section apply; or

(ii) The pertinent criteria in §50.24 of this chapter for such an investigation to begin or continue are not submitted or not satisfied.

(c) *Discussion of deficiency.* Whenever FDA concludes that a deficiency exists in a clinical investigation that may be grounds for the imposition of clinical hold FDA will, unless patients are exposed to immediate and serious risk, attempt to discuss and satisfactorily resolve the matter with the sponsor before issuing the clinical hold order.

(d) *Imposition of clinical hold.* The clinical hold order may be made by telephone or other means of rapid communication or in writing. The clinical hold order will identify the studies under the IND to which the hold applies, and will briefly explain the basis for the action. The clinical hold order will be made by or on behalf of the Division Director with responsibility for review of the IND. As soon as possible, and no more than 30 days after imposition of the clinical hold, the Division Director will provide the sponsor a written explanation of the basis for the hold.

(e) *Resumption of clinical investigations.* If, by the terms of the clinical hold order, resumption of the affected investigation is permitted without prior notification by FDA once a stated correction or modification is made, the investigation may proceed as soon as the correction or modification is made. In all other cases, an investigation may only resume after the Division Director (or the Director's designee) with responsibility for review of the IND has notified the sponsor that the investigation may proceed. In these cases resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfied the agency that the investigation(s) can proceed. Resumption of a study may be authorized by telephone or other means of rapid communication.

(f) *Appeal.* If the sponsor disagrees with the reasons cited for the clinical hold, the sponsor may request reconsideration of the decision in accordance with §312.48.

(g) *Conversion of IND on clinical hold to inactive status.* If all investigations covered by an IND remain on clinical hold for 1 year or more, the IND may be placed on inactive status by FDA under §312.45.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19477, May 22, 1987; 57 FR 13249, Apr. 15, 1992; 61 FR 51530, Oct. 2, 1996]

§ 312.44 Termination.

(a) *General.* This section describes the procedures under which FDA may terminate an IND. If an IND is terminated, the sponsor shall end all clinical investigations conducted under the IND and recall or otherwise provide for the disposition of all unused supplies of the drug. A termination action may be based on deficiencies in the IND or in the conduct of an investigation under an IND. Except as provided in paragraph (d) of this section, a termination shall be preceded by a proposal to terminate by FDA and an opportunity for the sponsor to respond. FDA will, in general, only initiate an action under this section after first attempting to resolve differences informally or, when appropriate, through the clinical hold procedures described in § 312.42.

(b) *Grounds for termination*—(1) *Phase 1.* FDA may propose to terminate an IND during Phase 1 if it finds that:

(i) Human subjects would be exposed to an unreasonable and significant risk of illness or unjury.

(ii) The IND does not contain sufficient information required under § 312.23 to assess the safety to subjects of the clinical investigations.

(iii) The methods, facilities, and controls used for the manufacturing, processing, and packing of the investigational drug are inadequate to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for subject safety.

(iv) The clinical investigations are being conducted in a manner substantially different than that described in the protocols submitted in the IND.

(v) The drug is being promoted or distributed for commercial purposes not justified by the requirements of the investigation or permitted by § 312.7.

(vi) The IND, or any amendment or report to the IND, contains an untrue statement of a material fact or omits material information required by this part.

(vii) The sponsor fails promptly to investigate and inform the Food and Drug Administration and all investigators of serious and unexpected adverse experiences in accordance with § 312.32 or fails to make any other report required under this part.

(viii) The sponsor fails to submit an accurate annual report of the investigations in accordance with § 312.33.

(ix) The sponsor fails to comply with any other applicable requirement of this part, part 50, or part 56.

(x) The IND has remained on inactive status for 5 years or more.

(xi) The sponsor fails to delay a proposed investigation under the IND or to suspend an ongoing investigation that has been placed on clinical hold under § 312.42(b)(4).

(2) *Phase 2 or 3.* FDA may propose to terminate an IND during Phase 2 or Phase 3 if FDA finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(xi) of this section apply; or

(ii) The investigational plan or protocol(s) is not reasonable as a bona fide scientific plan to determine whether or not the drug is safe and effective for use; or

(iii) There is convincing evidence that the drug is not effective for the purpose for which it is being investigated.

(3) FDA may propose to terminate a treatment IND if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (x) of this section apply; or

(ii) Any of the conditions in § 312.42(b)(3) apply.

(c) *Opportunity for sponsor response.* (1) If FDA proposes to terminate an IND, FDA will notify the sponsor in writing, and invite correction or explanation within a period of 30 days.

(2) On such notification, the sponsor may provide a written explanation or correction or may request a conference with FDA to provide the requested explanation or correction. If the sponsor does not respond to the notification within the allocated time, the IND shall be terminated.

(3) If the sponsor responds but FDA does not accept the explanation or correction submitted, FDA shall inform the sponsor in writing of the reason for the nonacceptance and provide the sponsor with an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be terminated. The sponsor's request for a regulatory hearing must be made within 10 days of the sponsor's

receipt of FDA's notification of nonacceptance.

(d) *Immediate termination of IND.* Notwithstanding paragraphs (a) through (c) of this section, if at any time FDA concludes that continuation of the investigation presents an immediate and substantial danger to the health of individuals, the agency shall immediately, by written notice to the sponsor from the Director of the Center for Drug Evaluation and Research or the Director of the Center for Biologics Evaluation and Research, terminate the IND. An IND so terminated is subject to reinstatement by the Director on the basis of additional submissions that eliminate such danger. If an IND is terminated under this paragraph, the agency will afford the sponsor an opportunity for a regulatory hearing under part 16 on the question of whether the IND should be reinstated.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 57 FR 13249, Apr. 15, 1992]

§ 312.45 Inactive status.

(a) If no subjects are entered into clinical studies for a period of 2 years or more under an IND, or if all investigations under an IND remain on clinical hold for 1 year or more, the IND may be placed by FDA on inactive status. This action may be taken by FDA either on request of the sponsor or on FDA's own initiative. If FDA seeks to act on its own initiative under this section, it shall first notify the sponsor in writing of the proposed inactive status. Upon receipt of such notification, the sponsor shall have 30 days to respond as to why the IND should continue to remain active.

(b) If an IND is placed on inactive status, all investigators shall be so notified and all stocks of the drug shall be returned or otherwise disposed of in accordance with § 312.59.

(c) A sponsor is not required to submit annual reports to an IND on inactive status. An inactive IND is, however, still in effect for purposes of the public disclosure of data and information under § 312.130.

(d) A sponsor who intends to resume clinical investigation under an IND placed on inactive status shall submit a protocol amendment under § 312.30 containing the proposed general investigational plan for the coming year and appropriate protocols. If the protocol amendment relies on information previously submitted, the plan shall reference such information. Additional information supporting the proposed investigation, if any, shall be submitted in an information amendment. Notwithstanding the provisions of § 312.30, clinical investigations under an IND on inactive status may only resume (1) 30 days after FDA receives the protocol amendment, unless FDA notifies the sponsor that the investigations described in the amendment are subject to a clinical hold under § 312.42, or (2) on earlier notification by FDA that the clinical investigations described in the protocol amendment may begin.

(e) An IND that remains on inactive status for 5 years or more may be terminated under § 312.44.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.47 Meetings.

(a) *General.* Meetings between a sponsor and the agency are frequently useful in resolving questions and issues raised during the course of a clinical investigation. FDA encourages such meetings to the extent that they aid in the evaluation of the drug and in the solution of scientific problems concerning the drug, to the extent that FDA's resources permit. The general principle underlying the conduct of such meetings is that there should be free, full, and open communication about any scientific or medical question that may arise during the clinical investigation. These meetings shall be conducted and documented in accordance with part 10.

(b) *''End-of-Phase 2'' meetings and meetings held before submission of a marketing application.* At specific times during the drug investigation process, meetings between FDA and a sponsor can be especially helpful in minimizing wasteful expenditures of time and

money and thus in speeding the drug development and evaluation process. In particular, FDA has found that meetings at the end of Phase 2 of an investigation (end-of-Phase 2 meetings) are of considerable assistance in planning later studies and that meetings held near completion of Phase 3 and before submission of a marketing application (''pre-NDA'' meetings) are helpful in developing methods of presentation and submission of data in the marketing application that facilitate review and allow timely FDA response.

(1) *End-of-Phase 2 meetings*—(i) *Purpose.* The purpose of an end-of-Phase 2 meeting is to determine the safety of proceeding to Phase 3, to evaluate the Phase 3 plan and protocols, and to identify any additional information necessary to support a marketing application for the uses under investigation.

(ii) *Eligibility for meeting.* While the end-of-Phase 2 meeting is designed primarily for IND's involving new molecular entities or major new uses of marketed drugs, a sponsor of any IND may request and obtain an end-of-Phase 2 meeting.

(iii) *Timing.* To be most useful to the sponsor, end-of-Phase 2 meetings should be held before major commitments of effort and resources to specific Phase 3 tests are made. The scheduling of an end-of-Phase 2 meeting is not, however, intended to delay the transition of an investigation from Phase 2 to Phase 3.

(iv) *Advance information.* At least 1 month in advance of an end-of-Phase 2 meeting, the sponsor should submit background information on the sponsor's plan for Phase 3, including summaries of the Phase 1 and 2 investigations, the specific protocols for Phase 3 clinical studies, plans for any additional nonclinical studies, and, if available, tentative labeling for the drug. The recommended contents of such a submission are described more fully in FDA Staff Manual Guide 4850.7 that is publicly available under FDA's public information regulations in part 20.

(v) *Conduct of meeting.* Arrangements for an end-of-Phase 2 meeting are to be made with the division in FDA's Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which is responsible for review of the IND. The meeting will be scheduled by FDA at a time convenient to both FDA and the sponsor. Both the sponsor and FDA may bring consultants to the meeting. The meeting should be directed primarily at establishing agreement between FDA and the sponsor of the overall plan for Phase 3 and the objectives and design of particular studies. The adequacy of technical information to support Phase 3 studies and/or a marketing application may also be discussed. Agreements reached at the meeting on these matters will be recorded in minutes of the conference that will be taken by FDA in accordance with § 10.65 and provided to the sponsor. The minutes along with any other written material provided to the sponsor will serve as a permanent record of any agreements reached. Barring a significant scientific development that requires otherwise, studies conducted in accordance with the agreement shall be presumed to be sufficient in objective and design for the purpose of obtaining marketing approval for the drug.

(2) *''Pre-NDA'' meetings.* FDA has found that delays associated with the initial review of a marketing application may be reduced by exchanges of information about a proposed marketing application. The primary purpose of this kind of exchange is to uncover any major unresolved problems, to identify those studies that the sponsor is relying on as adequate and well-controlled to establish the drug's effectiveness, to acquaint FDA reviewers with the general information to be submitted in the marketing application (including technical information), to discuss appropriate methods for statistical analysis of the data, and to discuss the best approach to the presentation and formatting of data in the marketing application. Arrangements for such a meeting are to be initiated by the sponsor with the division responsible for review of the IND. To permit FDA to provide the sponsor with the most useful advice on preparing a marketing application, the sponsor should submit to FDA's reviewing division at least 1 month in advance of the meeting the following information:

(i) A brief summary of the clinical studies to be submitted in the application.

(ii) A proposed format for organizing the submission, including methods for presenting the data.

(iii) Any other information for discussion at the meeting.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990]

§ 312.48 Dispute resolution.

(a) *General.* The Food and Drug Administration is committed to resolving differences between sponsors and FDA reviewing divisions with respect to requirements for IND's as quickly and amicably as possible through the cooperative exchange of information and views.

(b) *Administrative and procedural issues.* When administrative or procedural disputes arise, the sponsor should first attempt to resolve the matter with the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND, beginning with the consumer safety officer assigned to the application. If the dispute is not resolved, the sponsor may raise the matter with the person designated as ombudsman, whose function shall be to investigate what has happened and to facilitate a timely and equitable resolution. Appropriate issues to raise with the ombudsman include resolving difficulties in scheduling meetings and obtaining timely replies to inquiries. Further details on this procedure are contained in FDA Staff Manual Guide 4820.7 that is publicly available under FDA's public information regulations in part 20.

(c) *Scientific and medical disputes.* (1) When scientific or medical disputes arise during the drug investigation process, sponsors should discuss the matter directly with the responsible reviewing officials. If necessary, sponsors may request a meeting with the appropriate reviewing officials and management representatives in order to seek a resolution. Requests for such meetings shall be directed to the director of the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times.

(2) The ''end-of-Phase 2'' and ''pre-NDA'' meetings described in § 312.47(b) will also provide a timely forum for discussing and resolving scientific and medical issues on which the sponsor disagrees with the agency.

(3) In requesting a meeting designed to resolve a scientific or medical dispute, applicants may suggest that FDA seek the advice of outside experts, in which case FDA may, in its discretion, invite to the meeting one or more of its advisory committee members or other consultants, as designated by the agency. Applicants may rely on, and may bring to any meeting, their own consultants. For major scientific and medical policy issues not resolved by informal meetings, FDA may refer the matter to one of its standing advisory committees for its consideration and recommendations.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990]

Subpart D—Responsibilities of Sponsors and Investigators

§ 312.50 General responsibilities of sponsors.

Sponsors are responsibile for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the investigations, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug. Additional specific responsibilities of sponsors are described elsewhere in this part.

§ 312.52 Transfer of obligations to a contract research organization.

(a) A sponsor may transfer responsibility for any or all of the obligations set forth in this part to a contract research organization. Any such transfer shall be described in writing. If not all obligations are transferred, the writing is required to describe each of the obligations being assumed by the contract research organization. If all obligations are transferred, a general statement that all obligations have been transferred is acceptable. Any obligation not covered by the written description shall be deemed not to have been transferred.

(b) A contract research organization that assumes any obligation of a sponsor shall comply with the specific regulations in this chapter applicable to this obligation and shall be subject to the same regulatory action as a sponsor for failure to comply with any obligation assumed under these regulations. Thus, all references to ''sponsor'' in this part apply to a contract research organization to the extent that it assumes one or more obligations of the sponsor.

§ 312.53 Selecting investigators and monitors.

(a) *Selecting investigators.* A sponsor shall select only investigators qualified by training and experience as appropriate experts to investigate the drug.

(b) *Control of drug.* A sponsor shall ship investigational new drugs only to investigators participating in the investigation.

(c) *Obtaining information from the investigator.* Before permitting an investigator to begin participation in an investigation, the sponsor shall obtain the following:

(1) A signed investigator statement (Form FDA–1572) containing:

(i) The name and address of the investigator;

(ii) The name and code number, if any, of the protocol(s) in the IND identifying the study(ies) to be conducted by the investigator;

(iii) The name and address of any medical school, hospital, or other research facility where the clinical investigation(s) will be conducted;

(iv) The name and address of any clinical laboratory facilities to be used in the study;

(v) The name and address of the IRB that is responsible for review and approval of the study(ies);

(vi) A commitment by the investigator that he or she:

(*a*) Will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, the rights, or welfare of subjects;

(*b*) Will comply with all requirements regarding the obligations of clinical investigators and all other pertinent requirements in this part;

(*c*) Will personally conduct or supervise the described investigation(s);

(*d*) Will inform any potential subjects that the drugs are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent (21 CFR part 50) and institutional review board review and approval (21 CFR part 56) are met;

(*e*) Will report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with § 312.64;

(*f*) Has read and understands the information in the investigator's brochure, including the potential risks and side effects of the drug; and

(*g*) Will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

(vii) A commitment by the investigator that, for an investigation subject to an institutional review requirement under part 56, an IRB that complies with the requirements of that part will be responsible for the initial and continuing review and approval of the clinical investigation and that the investigator will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to the human subjects.

(viii) A list of the names of the subinvestigators (e.g., research fellows, residents) who will be assisting the investigator in the conduct of the investigation(s).

(2) *Curriculum vitae.* A curriculum vitae or other statement of qualifications of the investigator showing the education, training, and experience that qualifies the investigator as an expert in the clinical investigation of the drug for the use under investigation.

(3) *Clinical protocol.* (i) For Phase 1 investigations, a general outline of the planned investigation including the estimated duration of the study and the maximum number of subjects that will be involved.

(ii) For Phase 2 or 3 investigations, an outline of the study protocol including an approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; the clinical uses to be investigated; characteristics of subjects by age, sex, and condition; the kind of clinical observations and laboratory tests to be conducted; the estimated duration of the study; and copies or a description of case report forms to be used.

(4) *Financial disclosure information.* Sufficient accurate financial information to allow the sponsor to submit complete and accurate certification or disclosure statements required under part 54 of this chapter. The sponsor shall obtain a commitment from the clinical investigator to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(d) *Selecting monitors.* A sponsor shall select a monitor qualified by training and experience to monitor the progress of the investigation.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 312.53 was amended by adding new paragraph (c)(4), effective Feb. 2, 1999.

§ 312.54 Emergency research under § 50.24 of this chapter.

(a) The sponsor shall monitor the progress of all investigations involving an exception from informed consent under § 50.24 of this chapter. When the sponsor receives from the IRB information concerning the public disclosures required by § 50.24(a)(7)(ii) and (a)(7)(iii) of this chapter, the sponsor promptly shall submit to the IND file and to Docket Number 95S–0158 in the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857, copies of the information that was disclosed, identified by the IND number.

(b) The sponsor also shall monitor such investigations to identify when an IRB determines that it cannot approve the research because it does not meet the criteria in the exception in § 50.24(a) of this chapter or because of other relevant ethical concerns. The sponsor promptly shall provide this information in writing to FDA, investigators who are asked to participate in this or a substantially equivalent clinical investigation, and other IRB's that are asked to review this or a substantially equivalent investigation.

[61 FR 51530, Oct. 2, 1996]

§ 312.55 Informing investigators.

(a) Before the investigation begins, a sponsor (other than a sponsor-investigator) shall give each participating clinical investigator an investigator brochure containing the information described in § 312.23(a)(5).

(b) The sponsor shall, as the overall investigation proceeds, keep each participating investigator informed of new observations discovered by or reported to the sponsor on the drug, particularly with respect to adverse effects and safe use. Such information may be distributed to investigators by means of periodically revised investigator brochures, reprints or published studies, reports or letters to clinical investigators, or other appropriate means.

Important safety information is required to be relayed to investigators in accordance with § 312.32.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.56 Review of ongoing investigations.

(a) The sponsor shall monitor the progress of all clinical investigations being conducted under its IND.

(b) A sponsor who discovers that an investigator is not complying with the signed agreement (Form FDA–1572), the general investigational plan, or the requirements of this part or other applicable parts shall promptly either secure compliance or discontinue shipments of the investigational new drug to the investigator and end the investigator's participation in the investigation. If the investigator's participation in the investigation is ended, the sponsor shall require that the investigator dispose of or return the investigational drug in accordance with the requirements of § 312.59 and shall notify FDA.

(c) The sponsor shall review and evaluate the evidence relating to the safety and effectiveness of the drug as it is obtained from the investigator. The sponsors shall make such reports to FDA regarding information relevant to the safety of the drug as are required under § 312.32. The sponsor shall make annual reports on the progress of the investigation in accordance with § 312.33.

(d) A sponsor who determines that its investigational drug presents an unreasonable and significant risk to subjects shall discontinue those investigations that present the risk, notify FDA, all institutional review boards, and all investigators who have at any time participated in the investigation of the discontinuance, assure the disposition of all stocks of the drug outstanding as required by § 312.59, and furnish FDA with a full report of the sponsor's actions. The sponsor shall discontinue the investigation as soon as possible, and in no event later than 5 working days after making the determination that the investigation should be discontinued. Upon request, FDA will confer with a sponsor on the need to discontinue an investigation.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.57 Recordkeeping and record retention.

(a) A sponsor shall maintain adequate records showing the receipt, shipment, or other disposition of the investigational drug. These records are required to include, as appropriate, the name of the investigator to whom the drug is shipped, and the date, quantity, and batch or code mark of each such shipment.

(b) A sponsor shall maintain complete and accurate records showing any financial interest in § 54.4(a)(3)(i), (a)(3)(ii), (a)(3)(iii), and (a)(3)(iv) of this chapter paid to clinical investigators by the sponsor of the covered study. A sponsor shall also maintain complete and accurate records concerning all other financial interests of investigators subject to part 54 of this chapter.

(c) A sponsor shall retain the records and reports required by this part for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

(d) A sponsor shall retain reserve samples of any test article and reference standard identified in, and used in any of the bioequivalence or bioavailability studies described in, § 320.38 or § 320.63 of this chapter, and release the reserve samples to FDA upon request, in accordance with, and for the period specified in § 320.38.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 58 FR 25926, Apr. 28, 1993; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 312.57 was amended by redesignating paragraphs (b) and (c) as paragraphs (c) and

(d) and by adding a new paragraph (b), effective Feb. 2, 1999.

§ 312.58 Inspection of sponsor's records and reports.

(a) *FDA inspection.* A sponsor shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify any records and reports relating to a clinical investigation conducted under this part. Upon written request by FDA, the sponsor shall submit the records or reports (or copies of them) to FDA. The sponsor shall discontinue shipments of the drug to any investigator who has failed to maintain or make available records or reports of the investigation as required by this part.

(b) *Controlled substances.* If an investigational new drug is a substance listed in any schedule of the Controlled Substances Act (21 U.S.C. 801; 21 CFR part 1308), records concerning shipment, delivery, receipt, and disposition of the drug, which are required to be kept under this part or other applicable parts of this chapter shall, upon the request of a properly authorized employee of the Drug Enforcement Administration of the U.S. Department of Justice, be made available by the investigator or sponsor to whom the request is made, for inspection and copying. In addition, the sponsor shall assure that adequate precautions are taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.59 Disposition of unused supply of investigational drug.

The sponsor shall assure the return of all unused supplies of the investigational drug from each individual investigator whose participation in the investigation is discontinued or terminated. The sponsor may authorize alternative disposition of unused supplies of the investigational drug provided this alternative disposition does not expose humans to risks from the drug. The sponsor shall maintain written records of any disposition of the drug in accordance with § 312.57.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.60 General responsibilities of investigators.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation. An investigator shall, in accordance with the provisions of part 50 of this chapter, obtain the informed consent of each human subject to whom the drug is administered, except as provided in §§ 50.23 or 50.24 of this chapter. Additional specific responsibilities of clinical investigators are set forth in this part and in parts 50 and 56 of this chapter.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51530, Oct. 2, 1996]

§ 312.61 Control of the investigational drug.

An investigator shall administer the drug only to subjects under the investigator's personal supervision or under the supervision of a subinvestigator responsible to the investigator. The investigator shall not supply the investigational drug to any person not authorized under this part to receive it.

§ 312.62 Investigator recordkeeping and record retention.

(a) *Disposition of drug.* An investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies of the drug to the sponsor, or otherwise provide for disposition of the unused supplies of the drug under § 312.59.

(b) *Case histories.* An investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

(c) *Record retention.* An investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996]

§ 312.64 Investigator reports.

(a) *Progress reports.* The investigator shall furnish all reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained. The sponsor is required under § 312.33 to submit annual reports to FDA on the progress of the clinical investigations.

(b) *Safety reports.* An investigator shall promptly report to the sponsor any adverse effect that may reasonably be regarded as caused by, or probably caused by, the drug. If the adverse effect is alarming, the investigator shall report the adverse effect immediately.

(c) *Final report.* An investigator shall provide the sponsor with an adequate report shortly after completion of the investigator's participation in the investigation.

(d) *Financial disclosure reports.* The clinical investigator shall provide the sponsor with sufficient accurate financial information to allow an applicant to submit complete and accurate certification or disclosure statements as required under part 54 of this chapter. The clinical investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 312.64 was amended by adding new paragraph (d), effective Feb. 2, 1999.

§ 312.66 Assurance of IRB review.

An investigator shall assure that an IRB that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of the proposed clinical study. The investigator shall also assure that he or she will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others, and that he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.68 Inspection of investigator's records and reports.

An investigator shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the investigator pursuant to § 312.62. The investigator is not required to divulge subject names unless the records of particular individuals require a more detailed study of the cases, or unless

there is reason to believe that the records do not represent actual case studies, or do not represent actual results obtained.

§ 312.69 Handling of controlled substances.

If the investigational drug is subject to the Controlled Substances Act, the investigator shall take adequate precautions, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.70 Disqualification of a clinical investigator.

(a) If FDA has information indicating that an investigator (including a sponsor-investigator) has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has submitted to FDA or to the sponsor false information in any required report, the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research will furnish the investigator written notice of the matter complained of and offer the investigator an opportunity to explain the matter in writing, or, at the option of the investigator, in an informal conference. If an explanation is offered but not accepted by the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research, the investigator will be given an opportunity for a regulatory hearing under part 16 on the question of whether the investigator is entitled to receive investigational new drugs.

(b) After evaluating all available information, including any explanation presented by the investigator, if the Commissioner determines that the investigator has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has deliberately or repeatedly submitted false information to FDA or to the sponsor in any required report, the Commissioner will notify the investigator and the sponsor of any investigation in which the investigator has been named as a participant that the investigator is not entitled to receive investigational drugs. The notification will provide a statement of basis for such determination.

(c) Each IND and each approved application submitted under part 314 containing data reported by an investigator who has been determined to be ineligible to receive investigational drugs will be examined to determine whether the investigator has submitted unreliable data that are essential to the continuation of the investigation or essential to the approval of any marketing application.

(d) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the data remaining are inadequate to support a conclusion that it is reasonably safe to continue the investigation, the Commissioner will notify the sponsor who shall have an opportunity for a regulatory hearing under part 16. If a danger to the public health exists, however, the Commissioner shall terminate the IND immediately and notify the sponsor of the determination. In such case, the sponsor shall have an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be reinstated.

(e) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the continued approval of the drug product for which the data were submitted cannot be justified, the Commissioner will proceed to withdraw approval of the drug product in accordance with the applicable provisions of the act.

(f) An investigator who has been determined to be ineligible to receive investigational drugs may be reinstated as eligible when the Commissioner determines that the investigator has presented adequate assurances that the investigator will employ investigatioal drugs solely in compliance with the

provisions of this part and of parts 50 and 56.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 62 FR 46876, Sept. 5, 1997]

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

AUTHORITY: Secs. 501, 502, 503, 505, 506, 507, 701, 52 Stat. 1049–1053 as amended, 1055–1056 as amended, 55 Stat. 851, 59 Stat. 463 as amended (21 U.S.C. 351, 352, 353, 355, 356, 357, 371); sec. 351, 58 Stat. 702 as amended (42 U.S.C. 262); 21 CFR 5.10, 5.11.

SOURCE: 53 FR 41523, Oct. 21, 1988, unless otherwise noted.

§ 312.80 Purpose.

The purpose of this section is to establish procedures designed to expedite the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely-debilitating illnesses, especially where no satisfactory alternative therapy exists. As stated § 314.105(c) of this chapter, while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness. These procedures reflect the recognition that physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses, than they would accept from products that treat less serious illnesses. These procedures also reflect the recognition that the benefits of the drug need to be evaluated in light of the severity of the disease being treated. The procedure outlined in this section should be interpreted consistent with that purpose.

§ 312.81 Scope.

This section applies to new drug, antibiotic, and biological products that are being studied for their safety and effectiveness in treating life-threatening or severely-debilitating diseases.

(a) For purposes of this section, the term ''life-threatening'' means:

(1) Diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted; and

(2) Diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival.

(b) For purposes of this section, the term ''severely debilitating'' means diseases or conditions that cause major irreversible morbidity.

(c) Sponsors are encouraged to consult with FDA on the applicability of these procedures to specific products.

§ 312.82 Early consultation.

For products intended to treat life-threatening or severely-debilitating illnesses, sponsors may request to meet with FDA-reviewing officials early in the drug development process to review and reach agreement on the design of necessary preclinical and clinical studies. Where appropriate, FDA will invite to such meetings one or more outside expert scientific consultants or advisory committee members. To the extent FDA resources permit, agency reviewing officials will honor requests for such meetings

(a) *Pre-investigational new drug (IND) meetings.* Prior to the submission of the initial IND, the sponsor may request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of animal studies needed to initiate human testing. The meeting may also provide an opportunity for discussing the scope and design of phase 1 testing, and the best approach for presentation and formatting of data in the IND.

(b) *End-of-phase 1 meetings.* When data from phase 1 clinical testing are available, the sponsor may again request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of phase 2 controlled clinical

trials, with the goal that such testing will be adequate to provide sufficient data on the drug's safety and effectiveness to support a decision on its approvability for marketing. The procedures outlined in § 312.47(b)(1) with respect to end-of-phase 2 conferences, including documentation of agreements reached, would also be used for end-of-phase 1 meetings.

§ 312.83 Treatment protocols.

If the preliminary analysis of phase 2 test results appears promising, FDA may ask the sponsor to submit a treatment protocol to be reviewed under the procedures and criteria listed in §§ 312.34 and 312.35. Such a treatment protocol, if requested and granted, would normally remain in effect while the complete data necessary for a marketing application are being assembled by the sponsor and reviewed by FDA (unless grounds exist for clinical hold of ongoing protocols, as provided in § 312.42(b)(3)(ii)).

§ 312.84 Risk-benefit analysis in review of marketing applications for drugs to treat life-threatening and severely-debilitating illnesses.

(a) FDA's application of the statutory standards for marketing approval shall recognize the need for a medical risk-benefit judgment in making the final decision on approvability. As part of this evaluation, consistent with the statement of purpose in § 312.80, FDA will consider whether the benefits of the drug outweigh the known and potential risks of the drug and the need to answer remaining questions about risks and benefits of the drug, taking into consideration the severity of the disease and the absence of satisfactory alternative therapy.

(b) In making decisions on whether to grant marketing approval for products that have been the subject of an end-of-phase 1 meeting under § 312.82, FDA will usually seek the advice of outside expert scientific consultants or advisory committees. Upon the filing of such a marketing application under § 314.101 or part 601 of this chapter, FDA will notify the members of the relevant standing advisory committee of the application's filing and its availability for review.

(c) If FDA concludes that the data presented are not sufficient for marketing approval, FDA will issue (for a drug) a not approvable letter pursuant to § 314.120 of this chapter, or (for a biologic) a deficiencies letter consistent with the biological product licensing procedures. Such letter, in describing the deficiencies in the application, will address why the results of the research design agreed to under § 312.82, or in subsequent meetings, have not provided sufficient evidence for marketing approval. Such letter will also describe any recommendations made by the advisory committee regarding the application.

(d) Marketing applications submitted under the procedures contained in this section will be subject to the requirements and procedures contained in part 314 or part 600 of this chapter, as well as those in this subpart.

§ 312.85 Phase 4 studies.

Concurrent with marketing approval, FDA may seek agreement from the sponsor to conduct certain post-marketing (phase 4) studies to delineate additional information about the drug's risks, benefits, and optimal use. These studies could include, but would not be limited to, studying different doses or schedules of administration than were used in phase 2 studies, use of the drug in other patient populations or other stages of the disease, or use of the drug over a longer period of time.

§ 312.86 Focused FDA regulatory research.

At the discretion of the agency, FDA may undertake focused regulatory research on critical rate-limiting aspects of the preclinical, chemical/manufacturing, and clinical phases of drug development and evaluation. When initiated, FDA will undertake such research efforts as a means for meeting a public health need in facilitating the development of therapies to treat life-threatening or severely debilitating illnesses.

§ 312.87 Active monitoring of conduct and evaluation of clinical trials.

For drugs covered under this section, the Commissioner and other agency officials will monitor the progress of the conduct and evaluation of clinical trials and be involved in facilitating their appropriate progress.

§ 312.88 Safeguards for patient safety.

All of the safeguards incorporated within parts 50, 56, 312, 314, and 600 of this chapter designed to ensure the safety of clinical testing and the safety of products following marketing approval apply to drugs covered by this section. This includes the requirements for informed consent (part 50 of this chapter) and institutional review boards (part 56 of this chapter). These safeguards further include the review of animal studies prior to initial human testing (§ 312.23), and the monitoring of adverse drug experiences through the requirements of IND safety reports (§ 312.32), safety update reports during agency review of a marketing application (§ 314.50 of this chapter), and postmarketing adverse reaction reporting (§ 314.80 of this chapter).

Subpart F—Miscellaneous

§ 312.110 Import and export requirements.

(a) *Imports.* An investigational new drug offered for import into the United States complies with the requirements of this part if it is subject to an IND that is in effect for it under § 312.40 and: (1) The consignee in the United States is the sponsor of the IND; (2) the consignee is a qualified investigator named in the IND; or (3) the consignee is the domestic agent of a foreign sponsor, is responsible for the control and distribution of the investigational drug, and the IND identifies the consignee and describes what, if any, actions the consignee will take with respect to the investigational drug.

(b) *Exports.* An investigational new drug intended for export from the United States complies with the requirements of this part as follows:

(1) If an IND is in effect for the drug under § 312.40 and each person who receives the drug is an investigator named in the application; or

(2) If FDA authorizes shipment of the drug for use in a clinical investigation. Authorization may be obtained as follows:

(i) Through submission to the International Affairs Staff (HFY–50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a written request from the person that seeks to export the drug. A request must provide adequate information about the drug to satisfy FDA that the drug is appropriate for the proposed investigational use in humans, that the drug will be used for investigational purposes only, and that the drug may be legally used by that consignee in the importing country for the proposed investigational use. The request shall specify the quantity of the drug to be shipped per shipment and the frequency of expected shipments. If FDA authorizes exportation under this paragraph, the agency shall concurrently notify the government of the importing country of such authorization.

(ii) Through submission to the International Affairs Staff (HFY–50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a formal request from an authorized official of the government of the country to which the drug is proposed to be shipped. A request must specify that the foreign government has adequate information about the drug and the proposed investigational use, that the drug will be used for investigational purposes only, and that the foreign government is satisfied that the drug may legally be used by the intended consignee in that country. Such a request shall specify the quantity of drug to be shipped per shipment and the frequency of expected shipments.

(iii) Authorization to export an investigational drug under paragraph (b)(2)(i) or (ii) of this section may be revoked by FDA if the agency finds that the conditions underlying its authorization are not longer met.

(3) This paragraph applies only where the drug is to be used for the purpose of clinical investigation.

(4) This paragraph does not apply to the export of an antibiotic drug product shipped in accordance with the provisions of section 801(d) of the act.

(5) This paragraph does not apply to the export of new drugs (including biological products) approved for export under section 802 of the act or section 351(h)(1)(A) of the Public Health Service Act.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.120 Foreign clinical studies not conducted under an IND.

(a) *Introduction.* This section describes the criteria for acceptance by FDA of foreign clinical studies not conducted under an IND. In general, FDA accepts such studies provided they are well designed, well conducted, performed by qualified investigators, and conducted in accordance with ethical principles acceptable to the world community. Studies meeting these criteria may be utilized to support clinical investigations in the United States and/or marketing approval. Marketing approval of a new drug or antibiotic drug based solely on foreign clinical data is governed by § 314.106.

(b) *Data submissions.* A sponsor who wishes to rely on a foreign clinical study to support an IND or to support an application for marketing approval shall submit to FDA the following information:

(1) A description of the investigator's qualifications;

(2) A description of the research facilities;

(3) A detailed summary of the protocol and results of the study, and, should FDA request, case records maintained by the investigator or additional background data such as hospital or other institutional records;

(4) A description of the drug substance and drug product used in the study, including a description of components, formulation, specifications, and bioavailability of the specific drug product used in the clinical study, if available; and

(5) If the study is intended to support the effectiveness of a drug product, information showing that the study is adequate and well controlled under § 314.126.

(c) *Conformance with ethical principles.* (1) Foreign clinical research is required to have been conducted in accordance with the ethical principles stated in the ''Declaration of Helsinki'' (see paragraph (c)(4) of this section) or the laws and regulations of the country in which the research was conducted, whichever represents the greater protection of the individual.

(2) For each foreign clinical study submitted under this section, the sponsor shall explain how the research conformed to the ethical principles contained in the ''Declaration of Helsinki'' or the foreign country's standards, whichever were used. If the foreign country's standards were used, the sponsor shall explain in detail how those standards differ from the ''Declaration of Helsinki'' and how they offer greater protection.

(3) When the research has been approved by an independent review committee, the sponsor shall submit to FDA documentation of such review and approval, including the names and qualifications of the members of the committee. In this regard, a ''review committee'' means a committee composed of scientists and, where practicable, individuals who are otherwise qualified (e.g., other health professionals or laymen). The investigator may not vote on any aspect of the review of his or her protocol by a review committee.

(4) The ''Declaration of Helsinki'' states as follows:

RECOMMENDATIONS GUIDING PHYSICIANS IN BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, ''The health of my patient will be my first consideration,'' and the International Code of Medical Ethics declares that, ''A physician shall act only in the patient's interest when providing medical care

which might have the effect of weakening the physical and mental condition of the patient.''

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give a consent, the minor's consent must be

obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient—including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 56 FR 22113, May 14, 1991]

§ 312.130 Availability for public disclosure of data and information in an IND.

(a) The existence of an investigational new drug application will not be disclosed by FDA unless it has previously been publicly disclosed or acknowledged.

(b) The availability for public disclosure of all data and information in an investigational new drug application for a new drug or antibiotic drug will be handled in accordance with the provisions established in § 314.430 for the confidentiality of data and information in applications submitted in part 314. The availability for public disclosure of all data and information in an investigational new drug application for a biological product will be governed by the provisions of §§ 601.50 and 601.51.

(c) Notwithstanding the provisions of § 314.430, FDA shall disclose upon request to an individual to whom an investigational new drug has been given a copy of any IND safety report relating to the use in the individual.

(d) The availability of information required to be publicly disclosed for investigations involving an exception from informed consent under § 50.24 of this chapter will be handled as follows: Persons wishing to request the publicly disclosable information in the IND that was required to be filed in Docket Number 95S–0158 in the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857, shall submit a request under the Freedom of Information Act.

[52 FR 8831, Mar. 19, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988, as amended at 61 FR 51530, Oct. 2, 1996]

§ 312.140 Address for correspondence.

(a) Except as provided in paragraph (b) of this section, a sponsor shall send an initial IND submission to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, Park Bldg., Rm. 214, 12420 Parklawn Dr., Rockville, MD 20852. On receiving the IND, FDA will inform the sponsor which one of the divisions in the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research is responsible for the IND. Amendments,

reports, and other correspondence relating to matters covered by the IND should be directed to the appropriate division. The outside wrapper of each submission shall state what is contained in the submission, for example, ''IND Application'', ''Protocol Amendment'', etc.

(b) Applications for the products listed below should be submitted to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. (1) Products subject to the licensing provisions of the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) or subject to part 600; (2) ingredients packaged together with containers intended for the collection, processing, or storage of blood or blood components; (3) urokinase products; (4) plasma volume expanders and hydroxyethyl starch for leukapheresis; and (5) coupled antibodies, i.e., products that consist of an antibody component coupled with a drug or radionuclide component in which both components provide a pharmacological effect but the biological component determines the site of action.

(c) All correspondence relating to biological products for human use which are also radioactive drugs shall be submitted to the Division of Oncology and Radiopharmaceutical Drug Products (HFD–150), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, except that applications for coupled antibodies shall be submitted in accordance with paragraph (b) of this section.

(d) All correspondence relating to export of an investigational drug under § 312.110(b)(2) shall be submitted to the International Affairs Staff (HFY–50), Office of Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990]

§ 312.145 Guidelines.

(a) FDA has made available guidelines under § 10.90(b) to help persons to comply with certain requirements of this part.

(b) The Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research maintain lists of guidelines that apply to the Centers' regulations. The lists state how a person can obtain a copy of each guideline. A request for a copy of the lists should be directed to the CDER Executive Secretariat Staff (HFD–8), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, for drug products, and the Congressional, Consumer, and International Affairs Staff (HFB–142), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, for biological products.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990; 56 FR 3776, Jan. 31, 1991; 57 FR 10814, Mar. 31, 1992]

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or In Vitro Tests

§ 312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

(a) *Authorization to ship.* (1)(i) A person may ship a drug intended solely for tests in vitro or in animals used only for laboratory research purposes if it is labeled as follows:

CAUTION: Contains a new drug for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(ii) A person may ship a biological product for investigational in vitro diagnostic use that is listed in § 312.2(b)(2)(ii) if it is labeled as follows:

CAUTION: Contains a biological product for investigational in vitro diagnostic tests only.

(2) A person shipping a drug under paragraph (a) of this section shall use due diligence to assure that the consignee is regularly engaged in conducting such tests and that the shipment of

the new drug will actually be used for tests in vitro or in animals used only for laboratory research.

(3) A person who ships a drug under paragraph (a) of this section shall maintain adequate records showing the name and post office address of the expert to whom the drug is shipped and the date, quantity, and batch or code mark of each shipment and delivery. Records of shipments under paragraph (a)(1)(i) of this section are to be maintained for a period of 2 years after the shipment. Records and reports of data and shipments under paragraph (a)(1)(ii) of this section are to be maintained in accordance with §312.57(b). The person who ships the drug shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify records required to be maintained under this section.

(b) *Termination of authorization to ship.* FDA may terminate authorization to ship a drug under this section if it finds that:

(1) The sponsor of the investigation has failed to comply with any of the conditions for shipment established under this section; or

(2) The continuance of the investigation is unsafe or otherwise contrary to the public interest or the drug is used for purposes other than bona fide scientific investigation. FDA will notify the person shipping the drug of its finding and invite immediate correction. If correction is not immediately made, the person shall have an opportunity for a regulatory hearing before FDA pursuant to part 16.

(c) *Disposition of unused drug.* The person who ships the drug under paragraph (a) of this section shall assure the return of all unused supplies of the drug from individual investigators whenever the investigation discontinues or the investigation is terminated. The person who ships the drug may authorize in writing alternative disposition of unused supplies of the drug provided this alternative disposition does not expose humans to risks from the drug, either directly or indirectly (e.g., through food-producing animals). The shipper shall maintain records of any alternative disposition.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988]

PART 314—APPLICATIONS FOR FDA APPROVAL TO MARKET A NEW DRUG OR AN ANTIBIOTIC DRUG

Subpart A—General Provisions

Sec.
314.1 Scope of this part.
314.2 Purpose.
314.3 Definitions.

Subpart B—Applications

314.50 Content and format of an application.
314.52 Notice of certification of invalidity or noninfringement of a patent.
314.53 Submission of patent information.
314.54 Procedure for submission of an application requiring investigations for approval of a new indication for, or other change from, a listed drug.
314.60 Amendments to an unapproved application.
314.65 Withdrawal by the applicant of an unapproved application.
314.70 Supplements and other changes to an approved application.
314.71 Procedures for submission of a supplement to an approved application.
314.72 Change in ownership of an application.
314.80 Postmarketing reporting of adverse drug experiences.
314.81 Other postmarketing reports.
314.90 Waivers.

Subpart C—Abbreviated Applications

314.92 Drug products for which abbreviated applications may be submitted.
314.93 Petition to request a change from a listed drug.
314.94 Content and format of an abbreviated application.
314.95 Notice of certification of invalidity or noninfringement of a patent.
314.96 Amendments to an unapproved abbreviated application.
314.97 Supplements and other changes to an approved abbreviated application.
314.98 Postmarketing reports.
314.99 Other responsibilities of an applicant of an abbreviated application.

Subpart D—FDA Action on Applications and Abbreviated Applications

314.100 Timeframes for reviewing applications and abbreviated applications.
314.101 Filing an application and an abbreviated antibiotic application and receiving an abbreviated new drug application.
314.102 Communications between FDA and applicants.
314.103 Dispute resolution.
314.104 Drugs with potential for abuse.
314.105 Approval of an application and an abbreviated application.
314.106 Foreign data.
314.107 Effective date of approval of a 505(b)(2) application or abbreviated new drug application under section 505(j) of the act.
314.108 New drug product exclusivity.
314.110 Approvable letter to the applicant.
314.120 Not approvable letter to the applicant.
314.122 Submitting an abbreviated application for, or a 505(j)(2)(C) petition that relies on, a listed drug that is no longer marketed.
314.125 Refusal to approve an application or abbreviated antibiotic application.
314.126 Adequate and well-controlled studies.
314.127 Refusal to approve an abbreviated new drug application.
314.150 Withdrawal of approval of an application or abbreviated application.
314.151 Withdrawal of approval of an abbreviated new drug application under section 505(j)(5) of the act.
314.152 Notice of withdrawal of approval of an application or abbreviated application for a new drug.
314.153 Suspension of approval of an abbreviated new drug application.
314.160 Approval of an application or abbreviated application for which approval was previously refused, suspended, or withdrawn.
314.161 Determination of reasons for voluntary withdrawal of a listed drug.
314.162 Removal of a drug product from the list.
314.170 Adulteration and misbranding of an approved drug.

Subpart E—Hearing Procedures for New Drugs

314.200 Notice of opportunity for hearing; notice of participation and request for hearing; grant or denial of hearing.
314.201 Procedure for hearings.
314.235 Judicial review.

Subpart F—Administrative Procedures for Antibiotics

314.300 Procedure for the issuance, amendment, or repeal of regulations.

Subpart G—Miscellaneous Provisions

314.410 Imports and exports of new drugs and antibiotics.
314.420 Drug master files.
314.430 Availability for public disclosure of data and information in an application or abbreviated application.
314.440 Addresses for applications and abbreviated applications.
314.445 Guidelines.

Subpart H—Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses

314.500 Scope.
314.510 Approval based on a surrogate endpoint or on an effect on a clinical endpoint other than survival or irreversible morbidity.
314.520 Approval with restrictions to assure safe use.
314.530 Withdrawal procedures.
314.540 Postmarketing safety reporting.
314.550 Promotional materials.
314.560 Termination of requirements.

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 371, 374, 379e.

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted.

Subpart A—General Provisions

§ 314.1 Scope of this part.

(a) This part sets forth procedures and requirements for the submission to, and the review by, the Food and Drug Administration of applications and abbreviated applications, as well as amendments, supplements, and postmarketing reports to them, by persons seeking or holding approval from FDA of the following:

(1) An application or abbreviated application under section 505 of the Federal Food, Drug, and Cosmetic Act to market a new drug.

(2) An application or abbreviated application under section 507 of the Federal Food, Drug, and Cosmetic Act to market an antibiotic drug.

(b) This part does not apply to drug products subject to licensing by FDA under the Public Health Service Act (58 Stat. 632 as amended (42 U.S.C. 201 *et seq.*)) and subchapter F of chapter I of title 21 of the Code of Federal Regulations.

(c) References in this part to regulations in the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

[50 FR 7493, Feb. 22, 1985, as amended at 57 FR 17981, Apr. 28, 1992]

§ 314.2 Purpose.

The purpose of this part is to establish an efficient and thorough drug review process in order to: (a) Facilitate the approval of drugs shown to be safe and effective; and (b) ensure the disapproval of drugs not shown to be safe and effective. These regulations are also intended to establish an effective system for FDA's surveillance of marketed drugs. These regulations shall be construed in light of these objectives.

§ 314.3 Definitions.

(a) The definitions and interpretations contained in section 201 of the act apply to those terms when used in this part.

(b) The following definitions of terms apply to this part:

Abbreviated application means the application described under § 314.94, including all amendments and supplements to the application. ''Abbreviated application'' applies to both an abbreviated new drug application and an abbreviated antibiotic application.

Act means the Federal Food, Drug, and Cosmetic Act (sections 201–901 (21 U.S.C. 301–392)).

Applicant means any person who submits an application or abbreviated application or an amendment or supplement to them under this part to obtain FDA approval of a new drug or an antibiotic drug and any person who owns an approved application or abbreviated application.

Application means the application described under § 314.50, including all amendements and supplements to the application.

505(b)(2) Application means an application submitted under section 505(b)(1) of the act for a drug for which the investigations described in section 505(b)(1)(A) of the act and relied upon by the applicant for approval of the application were not conducted by or for the applicant and for which the applicant has not obtained a right of reference or use from the person by or for whom the investigations were conducted.

Approvable letter means a written communication to an applicant from FDA stating that the agency will approve the application or abbreviated application if specific additional information or material is submitted or specific conditions are met. An approvable letter does not constitute approval of any part of an application or abbreviated application and does not permit marketing of the drug that is the subject of the application or abbreviated application.

Approval letter means a written communication to an applicant from FDA approving an application or an abbreviated application.

Drug product means a finished dosage form, for example, tablet, capsule, or solution, that contains a drug substance, generally, but not necessarily, in association with one or more other ingredients.

Drug substance means an active ingredient that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or any function of the human body, but does not include intermediates use in the synthesis of such ingredient.

FDA means the Food and Drug Administration.

Listed drug means a new drug product that has an effective approval under section 505(c) of the act for safety and effectiveness or under section 505(j) of the act, which has not been withdrawn or suspended under section 505(e)(1) through (e)(5) or (j)(5) of the act, and which has not been withdrawn from sale for what FDA has determined are reasons of safety or effectiveness. Listed drug status is evidenced by the drug product's identification as a drug with an effective approval in the current edition of FDA's ''Approved Drug Products with Therapeutic Equivalence Evaluations'' (the list) or any current supplement thereto, as a drug with an effective approval. A drug product is deemed to be a listed drug on the date of effective approval of the application or abbreviated application for that drug product.

Not approvable letter means a written communication to an applicant from FDA stating that the agency does not consider the application or abbreviated application approvable because one or more deficiencies in the application or abbreviated application preclude the agency from approving it.

Reference listed drug means the listed drug identified by FDA as the drug product upon which an applicant relies in seeking approval of its abbreviated application.

Right of reference or use means the authority to rely upon, and otherwise use, an investigation for the purpose of obtaining approval of an application, including the ability to make available the underlying raw data from the investigation for FDA audit, if necessary.

The list means the list of drug products with effective approvals published in the current edition of FDA's publication ''Approved Drug Products with Therapeutic Equivalence Evaluations'' and any current supplement to the publication.

[50 FR 7493, Feb. 22, 1985, as amended at 57 FR 17981, Apr. 28, 1992]

Subpart B—Applications

§ 314.50 Content and format of an application.

Applications and supplements to approved applications are required to be submitted in the form and contain the information, as appropriate for the particular submission, required under this section. Three copies of the application are required: An archival copy, a review copy, and a field copy. An application for a new chemical entity will generally contain an application form, an index, a summary, five or six technical sections, case report tabulations of patient data, case report forms, drug samples, and labeling. Other applications will generally contain only some of those items, and information will be limited to that needed to support the particular submission. These include an application of the type described in section 505(b)(2) of the act, an amendment, and a supplement. The application is required to contain reports of all investigations of the drug product sponsored by the applicant, and all other information about the drug pertinent to an evaluation of the application that is received or otherwise obtained by the applicant from any source. FDA will maintain guidelines on the format and content of applications to assist applicants in their preparation.

(a) *Application form.* The applicant shall submit a completed and signed application form that contains the following:

(1) The name and address of the applicant; the date of the application; the application number if previously issued (for example, if the application is a resubmission, an amendment, or a supplement); the name of the drug product, including its established, proprietary, code, and chemical names; the dosage form and strength; the route of administration; the identification numbers of all investigational new drug applications that are referenced in the application; the identification numbers of all drug master files and other applications under this part that are referenced in the application; and the drug product's proposed indications for use.

(2) A statement whether the submission is an original submission, a 505(b)(2) application, a resubmission, or a supplement to an application under § 314.70.

(3) A statement whether the applicant proposes to market the drug product as a prescription or an over-the-counter product.

(4) A check-list identifying what enclosures required under this section the applicant is submitting.

(5) The applicant, or the applicant's attorney, agent, or other authorized official shall sign the application. If the person signing the application does not reside or have a place of business within the United States, the application is required to contain the name and address of, and be countersigned by, an attorney, agent, or other authorized official who resides or maintains a place of business within the United States.

(b) *Index.* The archival copy of the application is required to contain a comprehensive index by volume number and page number to the summary under paragraph (c) of this section, the technical sections under paragraph (d)

of this section, and the supporting information under paragraph (f) of this section.

(c) *Summary.* (1) An application is required to contain a summary of the application in enough detail that the reader may gain a good general understanding of the data and information in the application, including an understanding of the quantitative aspects of the data. The summary is not required for supplements under § 314.70. Resubmissions of an application should contain an updated summary, as appropriate. The summary should discuss all aspects of the application, and synthesize the information into a well-structured and unified document. The summary should be written at approximately the level of detail required for publication in, and meet the editorial standards generally applied by, refereed scientific and medical journals. In addition to the agency personnel reviewing the summary in the context of their review of the application, FDA may furnish the summary to FDA advisory committee members and agency officials whose duties require an understanding of the application. To the extent possible, data in the summary should be presented in tabular and graphic forms. FDA has prepared a guideline under § 10.90(b) that provides information about how to prepare a summary. The summary required under this paragraph may be used by FDA or the applicant to prepare the Summary Basis of Approval document for public disclosure (under § 314.430(e)(2)(ii)) when the application is approved.

(2) The summary is required to contain the following information:

(i) The proposed text of the labeling for the drug, with annotations to the information in the summary and technical sections of the application that support the inclusion of each statement in the labeling, and, if the application is for a prescription drug, statements describing the reasons for omitting a section or subsection of the labeling format in § 201.57.

(ii) A statement identifying the pharmacologic class of the drug and a discussion of the scientific rationale for the drug, its intended use, and the potential clinical benefits of the drug product.

(iii) A brief description of the marketing history, if any, of the drug outside the United States, including a list of the countries in which the drug has been marketed, a list of any countries in which the drug has been withdrawn from marketing for any reason related to safety or effectiveness, and a list of countries in which applications for marketing are pending. The description is required to describe both marketing by the applicant and, if known, the marketing history of other persons.

(iv) A summary of the chemistry, manufacturing, and controls section of the application.

(v) A summary of the nonclinical pharmacology and toxicology section of the application.

(vi) A summary of the human pharmacokinetics and bioavailability section of the application.

(vii) A summary of the microbiology section of the application (for anti-infective drugs only).

(viii) A summary of the clinical data section of the application, including the results of statistical analyses of the clinical trials.

(ix) A concluding discussion that presents the benefit and risk considerations related to the drug, including a discussion of any proposed additional studies or surveillance the applicant intends to conduct postmarketing.

(d) *Technical sections.* The application is required to contain the technical sections described below. Each technical section is required to contain data and information in sufficient detail to permit the agency to make a knowledgeable judgment about whether to approve the application or whether grounds exist under section 505(d) or 507 of the act to refuse to approve the application. The required technical sections are as follows:

(1) *Chemistry, manufacturing, and controls section.* A section describing the composition, manufacture, and specification of the drug substance and the drug product, including the following:

(i) *Drug substance.* A full description of the drug substance including its physical and chemical characteristics and stability; the name and address of

its manufacturer; the method of synthesis (or isolation) and purification of the drug substance; the process controls used during manufacture and packaging; and such specifications and analytical methods as are necessary to assure the identity, strength, quality, and purity of the drug substance and the bioavailability of the drug products made from the substance, including, for example, specifications relating to stability, sterility, particle size, and crystalline form. The application may provide additionally for the use of alternatives to meet any of these requirements, including alternative sources, process controls, methods, and specifications. Reference to the current edition of the U.S. Pharmacopeia and the National Formulary may satisfy relevant requirements in this paragraph.

(ii)(*a*) *Drug product.* A list of all components used in the manufacture of the drug product (regardless of whether they appear in the drug product); and a statement of the composition of the drug product; a statement of the specifications and analytical methods for each component; the name and address of each manufacturer the drug product; a description of the manufacturing and packaging procedures and in-process controls for the drug product; such specifications and analytical methods as are necessary to assure the identity, strength, quality, purity, and bioavailability of the drug product, including, for example, specifications relating to sterility, dissolution rate, containers and closure systems; and stability data with proposed expiration dating. The application may provide additionally for the use of alternatives to meet any of these requirements, including alternative components, manufacturing and packaging procedures, in-process controls, methods, and specifications. Reference to the current edition of the U.S. Pharmacopeia and the National Formulary may satisfy relevant requirements in this paragraph.

(*b*) Unless provided by paragraph (d)(1)(ii)(*a*) of this section, for each batch of the drug product used to conduct a bioavailability or bioequivalence study described in § 320.38 or § 320.63 of this chapter or used to conduct a primary stability study: The batch production record; the specifications and test procedures for each component and for the drug product; the names and addresses of the sources of the active and noncompendial inactive components and of the container and closure system for the drug product; the name and address of each contract facility involved in the manufacture, processing, packaging, or testing of the drug product and identification of the operation performed by each contract facility; and the results of any test performed on the components used in the manufacture of the drug product as required by § 211.84(d) of this chapter and on the drug product as required by § 211.165 of this chapter.

(*c*) The proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product or a comparably detailed description of the production process for a representative batch of the drug product.

(iii) *Environmental impact.* The application is required to contain either a claim for categorical exclusion under § 25.30 or 25.31 of this chapter or an environmental assessment under § 25.40 of this chapter.

(iv) The applicant may, at its option, submit a complete chemistry, manufacturing, and controls section 90 to 120 days before the anticipated submission of the remainder of the application. FDA will review such early submissions as resources permit.

(v) Except for a foreign applicant, the applicant shall include a statement certifying that the field copy of the application has been provided to the applicant's home FDA district office.

(2) *Nonclinical pharmacology and toxicology section.* A section describing, with the aid of graphs and tables, animal and in vitro studies with drug, including the following:

(i) Studies of the pharmacological actions of the drug in relation to its proposed therapeutic indication and studies that otherwise define the pharmacologic properties of the drug or are pertinent to possible adverse effects.

(ii) Studies of the toxicological effects of the drug as they relate to the

drug's intended clinical uses, including, as appropriate, studies assessing the drug's acute, subacute, and chronic toxicity; carcinogenicity; and studies of toxicities related to the drug's particular mode of administration or conditions of use.

(iii) Studies, as appropriate, of the effects of the drug on reproduction and on the developing fetus.

(iv) Any studies of the absorption, distribution, metabolism, and excretion of the drug in animals.

(v) For each nonclinical laboratory study subject to the good laboratory practice regulations under part 58 a statement that it was conducted in compliance with the good laboratory practice regulations in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance.

(3) *Human pharmacokinetics and bioavailability section.* A section describing the human pharmacokinetic data and human bioavailability data, or information supporting a waiver of the submission of in vivo bioavailability data under subpart B of part 320, including the following:

(i) A description of each of the bioavailability and pharmacokinetic studies of the drug in humans performed by or on behalf of the applicant that includes a description of the analytical and statistical methods used in each study and a statement with respect to each study that it either was conducted in compliance with the institutional review board regulations in part 56, or was not subject to the regulations under § 56.104 or § 56.105, and that it was conducted in compliance with the informed consent regulations in part 50.

(ii) If the application describes in the chemistry, manufacturing, and controls section specifications or analytical methods needed to assure the bioavailability of the drug product or drug substance, or both, a statement in this section of the rationale for establishing the specification or analytical methods, including data and information supporting the rationale.

(iii) A summarizing discussion and analysis of the pharmacokinetics and metabolism of the active ingredients and the bioavailability or bioequivalence, or both, of the drug product.

(4) *Microbiology section.* If the drug is an anti-infective drug, a section describing the microbiology data, including the following:

(i) A description of the biochemical basis of the drug's action on microbial physiology.

(ii) A description of the antimicrobial spectra of the drug, including results of in vitro preclinical studies to demonstrate concentrations of the drug required for effective use.

(iii) A description of any known mechanisms of resistance to the drug, including results of any known epidemiologic studies to demonstrate prevalence of resistance factors.

(iv) A description of clinical microbiology laboratory methods (for example, in vitro sensitivity discs) needed for effective use of the drug.

(5) *Clinical data section.* A section describing the clinical investigations of the drug, including the following:

(i) A description and analysis of each clinical pharmacology study of the drug, including a brief comparison of the results of the human studies with the animal pharmacology and toxicology data.

(ii) A description and analysis of each controlled clinical study pertinent to a proposed use of the drug, including the protocol and a description of the statistical analyses used to evaluate the study. If the study report is an interim analysis, this is to be noted and a projected completion date provided. Controlled clinical studies that have not been analyzed in detail for any reason (e.g., because they have been discontinued or are incomplete) are to be included in this section, including a copy of the protocol and a brief description of the results and status of the study.

(iii) A description of each uncontrolled clinical study, a summary of the results, and a brief statement explaining why the study is classified as uncontrolled.

(iv) A description and analysis of any other data or information relevant to an evaluation of the safety and effectiveness of the drug product obtained or otherwise received by the applicant from any source, foreign or domestic, including information derived from

clinical investigations, including controlled and uncontrolled studies of uses of the drug other than those proposed in the application, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers.

(v) An integrated summary of the data demonstrating substantial evidence of effectiveness for the claimed indications. Evidence is also required to support the dosage and administration section of the labeling, including support for the dosage and dose interval recommended. The effectiveness data shall be presented by gender, age, and racial subgroups and shall identify any modifications of dose or dose interval needed for specific subgroups. Effectiveness data from other subgroups of the population of patients treated, when appropriate, such as patients with renal failure or patients with different levels of severity of the disease, also shall be presented.

(vi) A summary and updates of safety information, as follows:

(*a*) The applicant shall submit an integrated summary of all available information about the safety of the drug product, including pertinent animal data, demonstrated or potential adverse effects of the drug, clinically significant drug/drug interactions, and other safety considerations, such as data from epidemiological studies of related drugs. The safety data shall be presented by gender, age, and racial subgroups. When appropriate, safety data from other subgroups of the population of patients treated also shall be presented, such as for patients with renal failure or patients with different levels of severity of the disease. A description of any statistical analyses performed in analyzing safety data should also be included, unless already included under paragraph (d)(5)(ii) of this section.

(*b*) The applicant shall, under section 505(i) of the act, update periodically its pending application with new safety information learned about the drug that may reasonably affect the statement of contraindications, warnings, precautions, and adverse reactions in the draft labeling. These ''safety update reports'' are required to include the same kinds of information (from clinical studies, animal studies, and other sources) and are required to be submitted in the same format as the integrated summary in paragraph (d)(5)(vi)(*a*) of this section. In addition, the reports are required to include the case report forms for each patient who died during a clinical study or who did not complete the study because of an adverse event (unless this requirement is waived). The applicant shall submit these reports (*1*) 4 months after the initial submission; (*2*) following receipt of an approvable letter; and (*3*) at other times as requested by FDA. Prior to the submission of the first such report, applicants are encouraged to consult with FDA regarding further details on its form and content.

(vii) If the drug has a potential for abuse, a description and analysis of studies or information related to abuse of the drug, including a proposal for scheduling under the Controlled Substances Act. A description of any studies related to overdosage is also required, including information on dialysis, antidotes, or other treatments, if known.

(viii) An integrated summary of the benefits and risks of the drug, including a discussion of why the benefits exceed the risks under the conditions stated in the labeling.

(ix) A statement with respect to each clinical study involving human subjects that it either was conducted in compliance with the institutional review board regulations in part 56, or was not subject to the regulations under § 56.104 or § 56.105, and that it was conducted in compliance with the informed consent regulations in part 50.

(x) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer—in lieu of a listing of the specific obligations transferred—may be submitted.

(xi) If original subject records were audited or reviewed by the sponsor in the course of monitoring any clinical

study to verify the accuracy of the case reports submitted to the sponsor, a list identifying each clinical study so audited or reviewed.

(6) *Statistical section.* A section describing the statistical evaluation of clinical data, including the following:

(i) A copy of the information submitted under paragraph (d)(5)(ii) of this section concerning the description and analysis of each controlled clinical study, and the documentation and supporting statistical analyses used in evaluating the controlled clinical studies.

(ii) A copy of the information submitted under paragraph (d)(5)(vi)(*a*) of this section concerning a summary of information about the safety of the drug product, and the documentation and supporting statistical analyses used in evaluating the safety information.

(e) *Samples and labeling.* (1) Upon request from FDA, the applicant shall submit the samples described below to the places identified in the agency's request. FDA will generally ask applicants to submit samples directly to two or more agency laboratories that will perform all necessary tests on the samples and validate the applicant's analytical methods.

(i) Four representative samples of the following, each sample in sufficient quantity to permit FDA to perform three times each test described in the application to determine whether the drug substance and the drug product meet the specifications given in the application:

(*a*) The drug product proposed for marketing;

(*b*) The drug substance used in the drug product from which the samples of the drug product were taken; and

(*c*) Reference standards and blanks (except that reference standards recognized in an official compendium need not be submitted).

(ii) Samples of the finished market package, if requested by FDA.

(2) The applicant shall submit the following in the archival copy of the application:

(i) Three copies of the analytical methods and related descriptive information contained in the chemistry, manufacturing, and controls section under paragraph (d)(1) of this section for the drug substance and the drug product that are necessary for FDA's laboratories to perform all necessary tests on the samples and to validate the applicant's analytical methods. The related descriptive information includes a description of each sample; the proposed regulatory specifications for the drug; a detailed description of the methods of analysis; supporting data for accuracy, specificity, precision and ruggedness; and complete results of the applicant's tests on each sample.

(ii) Copies of the label and all labeling for the drug product (4 copies of draft labeling or 12 copies of final printed labeling).

(f) *Case report forms and tabulations.* The archival copy of the application is required to contain the following case report tabulations and case report forms:

(1) *Case report tabulations.* The application is required to contain tabulations of the data from each adequate and well-controlled study under § 314.126 (Phase 2 and Phase 3 studies as described in §§ 312.21 (b) and (c) of this chapter), tabulations of the data from the earliest clinical pharmacology studies (Phase 1 studies as described in § 312.21(a) of this chapter), and tabulations of the safety data from other clinical studies. Routine submission of other patient data from uncontrolled studies is not required. The tabulations are required to include the data on each patient in each study, except that the applicant may delete those tabulations which the agency agrees, in advance, are not pertinent to a review of the drug's safety or effectiveness. Upon request, FDA will discuss with the applicant in a ''pre-NDA'' conference those tabulations that may be appropriate for such deletion. Barring unforeseen circumstances, tabulations agreed to be deleted at such a conference will not be requested during the conduct of FDA's review of the application. If such unforeseen circumstances do occur, any request for deleted tabulations will be made by the director of the FDA division responsible for reviewing the application, in accordance with paragraph (f)(3) of this section.

(2) *Case report forms.* The application is required to contain copies of individual case report forms for each patient who died during a clinical study or who did not complete the study because of an adverse event, whether believed to be drug related or not, including patients receiving reference drugs or placebo. This requirement may be waived by FDA for specific studies if the case report forms are unnecessary for a proper review of the study.

(3) *Additional data.* The applicant shall submit to FDA additional case report forms and tabulations needed to conduct a proper review of the application, as requested by the director of the FDA division responsible for reviewing the application. The applicant's failure to submit information requested by FDA within 30 days after receipt of the request may result in the agency viewing any eventual submission as a major amendment under § 314.60 and extending the review period as necessary. If desired by the applicant, the FDA division director will verify in writing any request for additional data that was made orally.

(4) Applicants are invited to meet with FDA before submitting an application to discuss the presentation and format of supporting information. If the applicant and FDA agree, the applicant may submit tabulations of patient data and case report forms in a form other than hard copy, for example, on microfiche or computer tapes.

(g) *Other.* The following general requirements apply to the submission of information within the summary under paragraph (c) of this section and within the technical sections under paragraph (d) of this section.

(1) The applicant ordinarily is not required to resubmit information previously submitted, but may incorporate the information by reference. A reference to information submitted previously is required to identify the file by name, reference number, volume, and page number in the agency's records where the information can be found. A reference to information submitted to the agency by a person other than the applicant is required to contain a written statement that authorizes the reference and that is signed by the person who submitted the information.

(2) The applicant shall submit an accurate and complete English translation of each part of the application that is not in English. The applicant shall submit a copy of each original literature publication for which an English translation is submitted.

(3) If an applicant who submits a new drug application under section 505(b) of the act obtains a ''right of reference or use,'' as defined under § 314.3(b), to an investigation described in clause (A) of section 505(b)(1) of the act, the applicant shall include in its application a written statement signed by the owner of the data from each such investigation that the applicant may rely on in support of the approval of its application, and provide FDA access to, the underlying raw data that provide the basis for the report of the investigation submitted in its application.

(h) *Patent information.* The application is required to contain the patent information described under § 314.53.

(i) *Patent certification*—(1) *Contents.* A 505(b)(2) application is required to contain the following:

(i) *Patents claiming drug, drug product, or method of use.* (A) Except as provided in paragraph (i)(2) of this section, a certification with respect to each patent issued by the United States Patent and Trademark Office that, in the opinion of the applicant and to the best of its knowledge, claims a drug (the drug product or drug substance that is a component of the drug product) on which investigations that are relied upon by the applicant for approval of its application were conducted or that claims an approved use for such drug and for which information is required to be filed under section 505(b) and (c) of the act and § 314.53. For each such patent, the applicant shall provide the patent number and certify, in its opinion and to the best of its knowledge, one of the following circumstances:

(*1*) That the patent information has not been submitted to FDA. The applicant shall entitle such a certification ''Paragraph I Certification'';

(*2*) That the patent has expired. The applicant shall entitle such a certification ''Paragraph II Certification'';

(*3*) The date on which the patent will expire. The applicant shall entitle such a certification ''Paragraph III Certification''; or

(*4*) That the patent is invalid, unenforceable, or will not be infringed by the manufacture, use, or sale of the drug product for which the application is submitted. The applicant shall entitle such a certification ''Paragraph IV Certification''. This certification shall be submitted in the following form:

I, (*name of applicant*), certify that Patent No. _____ (*is invalid, unenforceable, or will not be infringed by the manufacture, use, or sale of*) (*name of proposed drug product*) for which this application is submitted.

The certification shall be accompanied by a statement that the applicant will comply with the requirements under § 314.52(a) with respect to providing a notice to each owner of the patent or their representatives and to the holder of the approved application for the drug product which is claimed by the patent or a use of which is claimed by the patent and with the requirements under § 314.52(c) with respect to the content of the notice.

(B) If the drug on which investigations that are relied upon by the applicant were conducted is itself a licensed generic drug of a patented drug first approved under section 505(b) of the act, the appropriate patent certification under this section with respect to each patent that claims the first-approved patented drug or that claims an approved use for such a drug.

(ii) *No relevant patents.* If, in the opinion of the applicant and to the best of its knowledge, there are no patents described in paragraph (i)(1)(i) of this section, a certification in the following form:

In the opinion and to the best knowledge of (*name of applicant*), there are no patents that claim the drug or drugs on which investigations that are relied upon in this application were conducted or that claim a use of such drug or drugs.

(iii) *Method of use patent.* (A) If information that is submitted under section 505(b) or (c) of the act and § 314.53 is for a method of use patent, and the labeling for the drug product for which the applicant is seeking approval does not include any indications that are covered by the use patent, a statement explaining that the method of use patent does not claim any of the proposed indications.

(B) If the labeling of the drug product for which the applicant is seeking approval includes an indication that, according to the patent information submitted under section 505(b) or (c) of the act and § 314.53 or in the opinion of the applicant, is claimed by a use patent, the applicant shall submit an applicable certification under paragraph (i)(1)(i) of this section.

(2) *Method of manufacturing patent.* An applicant is not required to make a certification with respect to any patent that claims only a method of manufacturing the drug product for which the applicant is seeking approval.

(3) *Licensing agreements.* If a 505(b)(2) application is for a drug or method of using a drug claimed by a patent and the applicant has a licensing agreement with the patent owner, the applicant shall submit a certification under paragraph (i)(1)(i)(A)(*4*) of this section (''Paragraph IV Certification'') as to that patent and a statement that it has been granted a patent license. If the patent owner consents to an immediate effective date upon approval of the 505(b)(2) application, the application shall contain a written statement from the patent owner that it has a licensing agreement with the applicant and that it consents to an immediate effective date.

(4) *Late submission of patent information.* If a patent described in paragraph (i)(1)(i)(A) of this section is issued and the holder of the approved application for the patented drug does not submit the required information on the patent within 30 days of issuance of the patent, an applicant who submitted a 505(b)(2) application that, before the submission of the patent information, contained an appropriate patent certification is not required to submit an amended certification. An applicant whose 505(b)(2) application is filed after a late submission of patent information or whose 505(b)(2) application was previously filed but did not contain an appropriate patent certification at the time of the patent submission shall submit a certification under paragraph (i)(1)(i) or (i)(1)(ii) of this section or a

statement under paragraph (i)(1)(iii) of this section as to that patent.

(5) *Disputed patent information.* If an applicant disputes the accuracy or relevance of patent information submitted to FDA, the applicant may seek a confirmation of the correctness of the patent information in accordance with the procedures under § 314.53(f). Unless the patent information is withdrawn or changed, the applicant must submit an appropriate certification for each relevant patent.

(6) *Amended certifications.* A certification submitted under paragraphs (i)(1)(i) through (i)(1)(iii) of this section may be amended at any time before the effective date of the approval of the application. An applicant shall submit an amended certification as an amendment to a pending application or by letter to an approved application. If an applicant with a pending application voluntarily makes a patent certification for an untimely filed patent, the applicant may withdraw the patent certification for the untimely filed patent. Once an amendment or letter for the change in certification has been submitted, the application will no longer be considered to be one containing the prior certification.

(i) *After finding of infringement.* An applicant who has submitted a certification under paragraph (i)(1)(i)(A)(*4*) of this section and is sued for patent infringement within 45 days of the receipt of notice sent under § 314.52 shall amend the certification if a final judgment in the action is entered finding the patent to be infringed unless the final judgment also finds the patent to be invalid. In the amended certification, the applicant shall certify under paragraph (i)(1)(i)(A)(*3*) of this section that the patent will expire on a specific date.

(ii) *After removal of a patent from the list.* If a patent is removed from the list, any applicant with a pending application (including a tentatively approved application with a delayed effective date) who has made a certification with respect to such patent shall amend its certification. The applicant shall certify under paragraph (i)(1)(ii) of this section that no patents described in paragraph (i)(1)(i) of this section claim the drug or, if other relevant patents claim the drug, shall amend the certification to refer only to those relevant patents. In the amendment, the applicant shall state the reason for the change in certification (that the patent is or has been removed from the list). A patent that is the subject of a lawsuit under § 314.107(c) shall not be removed from the list until FDA determines either that no delay in effective dates of approval is required under that section as a result of the lawsuit, that the patent has expired, or that any such period of delay in effective dates of approval is ended. An applicant shall submit an amended certification as an amendment to a pending application. Once an amendment for the change has been submitted, the application will no longer be considered to be one containing a certification under paragraph (i)(1)(i)(A)(*4*) of this section.

(iii) *Other amendments.* (A) Except as provided in paragraphs (i)(4) and (i)(6)(iii)(B) of this section, an applicant shall amend a submitted certification if, at any time before the effective date of the approval of the application, the applicant learns that the submitted certification is no longer accurate.

(B) An applicant is not required to amend a submitted certification when information on an otherwise applicable patent is submitted after the effective date of approval for the 505(b)(2) application.

(j) *Claimed exclusivity.* A new drug product, upon approval, may be entitled to a period of marketing exclusivity under the provisions of § 314.108. If an applicant believes its drug product is entitled to a period of exclusivity, it shall submit with the new drug application prior to approval the following information:

(1) A statement that the applicant is claiming exclusivity.

(2) A reference to the appropriate paragraph under § 314.108 that supports its claim.

(3) If the applicant claims exclusivity under § 314.108(b)(2), information to show that, to the best of its knowledge or belief, a drug has not previously been approved under section 505(b) of the act containing any active moiety

in the drug for which the applicant is seeking approval.

(4) If the applicant claims exclusivity under § 314.108(b)(4) or (b)(5), the following information to show that the application contains ''new clinical investigations'' that are ''essential to approval of the application or supplement'' and were ''conducted or sponsored by the applicant:''

(i) *''New clinical investigations.''* A certification that to the best of the applicant's knowledge each of the clinical investigations included in the application meets the definition of ''new clinical investigation'' set forth in § 314.108(a).

(ii) *''Essential to approval.''* A list of all published studies or publicly available reports of clinical investigations known to the applicant through a literature search that are relevant to the conditions for which the applicant is seeking approval, a certification that the applicant has thoroughly searched the scientific literature and, to the best of the applicant's knowledge, the list is complete and accurate and, in the applicant's opinion, such published studies or publicly available reports do not provide a sufficient basis for the approval of the conditions for which the applicant is seeking approval without reference to the new clinical investigation(s) in the application, and an explanation as to why the studies or reports are insufficient.

(iii) *''Conducted or sponsored by.''* If the applicant was the sponsor named in the Form FDA–1571 for an investigational new drug application (IND) under which the new clinical investigation(s) that is essential to the approval of its application was conducted, identification of the IND by number. If the applicant was not the sponsor of the IND under which the clinical investigation(s) was conducted, a certification that the applicant or its predecessor in interest provided substantial support for the clinical investigation(s) that is essential to the approval of its application, and information supporting the certification. To demonstrate ''substantial support,'' an applicant must either provide a certified statement from a certified public accountant that the applicant provided 50 percent or more of the cost of conducting the study or provide an explanation of why FDA should consider the applicant to have conducted or sponsored the study if the applicant's financial contribution to the study is less than 50 percent or the applicant did not sponsor the investigational new drug. A predecessor in interest is an entity, e.g., a corporation, that the applicant has taken over, merged with, or purchased, or from which the applicant has purchased all rights to the drug. Purchase of nonexclusive rights to a clinical investigation after it is completed is not sufficient to satisfy this definition.

(k) *Financial certification or disclosure statement.* The application shall contain a financial certification or disclosure statement or both as required by part 54 of this chapter.

(l) *Format of an original application.* (1) The applicant shall submit a complete archival copy of the application that contains the information required under paragraphs (a) through (f) of this section. FDA will maintain the archival copy during the review of the application to permit individual reviewers to refer to information that is not contained in their particular technical sections of the application, to give other agency personnel access to the application for official business, and to maintain in one place a complete copy of the application. An applicant may submit on microfiche the portions of the archival copy of the application described in paragraphs (b) through (d) of this section. Information relating to samples and labeling, described in paragraph (e) of this section, is required to be submitted in hard copy. Tabulations of patient data and case report forms, described in paragraph (f) of this section, may be submitted on microfiche only if the applicant and FDA agree. If FDA agrees, the applicant may use another suitable microform system.

(2) The applicant shall submit a review copy of the application. Each of the technical sections, described in paragraphs (d)(1) through (d)(6) of this section, in the review copy is required to be separately bound with a copy of the application form required under paragraph (a) of this section and a copy of the summary required under paragraph (c) of this section.

(3) The applicant shall submit a field copy of the application that contains the technical section described in paragraph (d)(1) of this section, a copy of the application form required under paragraph (a) of this section, a copy of the summary required under paragraph (c) of this section, and a certification that the field copy is a true copy of the technical section described in paragraph (d)(1) of this section contained in the archival and review copies of the application.

(4) The applicant may obtain from FDA sufficient folders to bind the archival, the review, and the field copies of the application.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 16668, Apr. 26, 1985; 50 FR 21238, May 23, 1985; 52 FR 8847, Mar. 19, 1987; 55 FR 11580, Mar. 29, 1990; 57 FR 17982, Apr. 28, 1992; 58 FR 47351, Sept. 8, 1993; 59 FR 13200, Mar. 21, 1994; 59 FR 50361, Oct. 3, 1994; 59 FR 60051, Nov. 21, 1994; 62 FR 40599, July 29, 1997; 63 FR 5252, Feb. 2, 1998; 63 FR 6862, Feb. 11, 1998]

EFFECTIVE DATE NOTE: 1. At 63 FR 5252, Feb. 2, 1998, § 314.50 was amended by redesignating paragraph (k) as paragraph (l) and by adding a new paragraph (k), effective Feb. 2, 1999.

2. At 63 FR 6862, Feb. 11, 1998, § 314.50 was amended by revising the second sentence and adding two new sentences after the second sentence in paragraph (d)(5)(v), and by adding two new sentences after the first sentence in paragraph (d)(5)(vi)(*a*), effective Aug. 10, 1998. For the convenience of the user, the superseded text is set forth as follows:

§ 314.50 Content and format of an application.

* * * * *

(d) * * *

(5) * * *

(v) * * * Evidence is also required to support the dosage and administration section of the labeling, including support for the dosage and dose interval recommended, and modifications for specific subgroups (for example, pediatrics, geriatrics, patients with renal failure).

* * * * *

§ 314.52 Notice of certification of invalidity or noninfringement of a patent.

(a) *Notice of certification.* For each patent which claims the drug or drugs on which investigations that are relied upon by the applicant for approval of its application were conducted or which claims a use for such drug or drugs and which the applicant certifies under § 314.50(i)(1)(i)(A)(*4*) that a patent is invalid, unenforceable, or will not be infringed, the applicant shall send notice of such certification by registered or certified mail, return receipt requested to each of the following persons:

(1) Each owner of the patent that is the subject of the certification or the representative designated by the owner to receive the notice. The name and address of the patent owner or its representative may be obtained from the United States Patent and Trademark Office; and

(2) The holder of the approved application under section 505(b) of the act for each drug product which is claimed by the patent or a use of which is claimed by the patent and for which the applicant is seeking approval, or, if the application holder does not reside or maintain a place of business within the United States, the application holder's attorney, agent, or other authorized official. The name and address of the application holder or its attorney, agent, or authorized official may be obtained from the Division of Drug Information Resources (HFD–80), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(3) This paragraph does not apply to a use patent that claims no uses for which the applicant is seeking approval.

(b) *Sending the notice.* The applicant shall send the notice required by paragraph (a) of this section when it receives from FDA an acknowledgment letter stating that its application has been filed. At the same time, the applicant shall amend its application to include a statement certifying that the notice has been provided to each person identified under paragraph (a) of this section and that the notice met the

content requirement under paragraph (c) of this section.

(c) *Content of a notice.* In the notice, the applicant shall cite section 505(b)(3)(B) of the act and shall include, but not be limited to, the following information:

(1) A statement that a 505(b)(2) application submitted by the applicant has been filed by FDA.

(2) The application number.

(3) The established name, if any, as defined in section 502(e)(3) of the act, of the proposed drug product.

(4) The active ingredient, strength, and dosage form of the proposed drug product.

(5) The patent number and expiration date, as submitted to the agency or as known to the applicant, of each patent alleged to be invalid, unenforceable, or not infringed.

(6) A detailed statement of the factual and legal basis of the applicant's opinion that the patent is not valid, unenforceable, or will not be infringed. The applicant shall include in the detailed statement:

(i) For each claim of a patent alleged not to be infringed, a full and detailed explanation of why the claim is not infringed.

(ii) For each claim of a patent alleged to be invalid or unenforceable, a full and detailed explanation of the grounds supporting the allegation.

(7) If the applicant does not reside or have a place of business in the United States, the name and address of an agent in the United States authorized to accept service of process for the applicant.

(d) *Amendment to an application.* If an application is amended to include the certification described in § 314.50(i), the applicant shall send the notice required by paragraph (a) of this section at the same time that the amendment to the application is submitted to FDA.

(e) *Documentation of receipt of notice.* The applicant shall amend its application to document receipt of the notice required under paragraph (a) of this section by each person provided the notice. The applicant shall include a copy of the return receipt or other similar evidence of the date the notification was received. FDA will accept as adequate documentation of the date of receipt a return receipt or a letter acknowledging receipt by the person provided the notice. An applicant may rely on another form of documentation only if FDA has agreed to such documentation in advance. A copy of the notice itself need not be submitted to the agency.

(f) *Approval.* If the requirements of this section are met, the agency will presume the notice to be complete and sufficient, and it will count the day following the date of receipt of the notice by the patent owner or its representative and by the approved application holder as the first day of the 45-day period provided for in section 505(c)(3)(C) of the act. FDA may, if the applicant amends its application with a written statement that a later date should be used, count from such later date.

[59 FR 50362, Oct. 3, 1994]

§ 314.53 Submission of patent information.

(a) *Who must submit patent information.* This section applies to any applicant who submits to FDA a new drug application or an amendment to it under section 505(b) of the act and § 314.50 or a supplement to an approved application under § 314.70, except as provided in paragraph (d)(2) of this section.

(b) *Patents for which information must be submitted.* An applicant described in paragraph (a) of this section shall submit information on each patent that claims the drug or a method of using the drug that is the subject of the new drug application or amendment or supplement to it and with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner of the patent engaged in the manufacture, use, or sale of the drug product. For purposes of this part, such patents consist of drug substance (ingredient) patents, drug product (formulation and composition) patents, and method of use patents. Process patents are not covered by this section and information on process patents may not be submitted to FDA. For patents that claim a drug substance or drug product, the applicant shall submit information only on those patents that claim a drug product that is the

subject of a pending or approved application, or that claim a drug substance that is a component of such a product. For patents that claim a method of use, the applicant shall submit information only on those patents that claim indications or other conditions of use of a pending or approved application.

(c) *Reporting requirements*—(1) *General requirements.* An applicant described in paragraph (a) of this section shall submit the following information for each patent described in paragraph (b) of this section:

(i) Patent number and the date on which the patent will expire.

(ii) Type of patent, i.e., drug, drug product, or method of use.

(iii) Name of the patent owner.

(iv) If the patent owner or applicant does not reside or have a place of business within the United States, the name of an agent (representative) of the patent owner or applicant who resides or maintains a place of business within the United States authorized to receive notice of patent certification under section 505(b)(3) and (j)(2)(B) of the act and §§ 314.52 and 314.95.

(2) *Formulation, composition, or method of use patents*—(i) *Original declaration.* For each formulation, composition, or method of use patent, in addition to the patent information described in paragraph (c)(1) of this section the applicant shall submit the following declaration:

The undersigned declares that Patent No. _____ covers the formulation, composition, and/or method of use of (*name of drug product*). This product is (*currently approved under section 505 of the Federal Food, Drug, and Cosmetic Act*) [or] (*the subject of this application for which approval is being sought):*

(ii) *Amendment of patent information upon approval.* Within 30 days after the date of approval of its application, if the application contained a declaration required under paragraph (c)(2)(i) of this section, the applicant shall by letter amend the declaration to identify each patent that claims the formulation, composition, or the specific indications or other conditions of use that have been approved.

(3) *No relevant patents.* If the applicant believes that there are no patents which claim the drug or the drug product or which claim a method of using the drug product and with respect to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner of the patent engaged in the manufacture, use, or sale of the drug product, it shall so declare.

(4) *Authorized signature.* The declarations required by this section shall be signed by the applicant or patent owner, or the applicant's or patent owner's attorney, agent (representative), or other authorized official.

(d) *When and where to submit patent information*—(1) *Original application.* An applicant shall submit with its original application submitted under this part, including an application described in section 505(b)(2) of the act, the information described in paragraph (c) of this section on each drug (ingredient), drug product (formulation and composition), and method of use patent issued before the application is filed with FDA and for which patent information is required to be submitted under this section. If a patent is issued after the application is filed with FDA but before the application is approved, the applicant shall, within 30 days of the date of issuance of the patent, submit the required patent information in an amendment to the application under § 314.60.

(2) *Supplements.* (i) An applicant shall submit patent information required under paragraph (c) of this section for a patent that claims the drug, drug product, or method of use for which approval is sought in any of the following supplements:

(A) To change the formulation;

(B) To add a new indication or other condition of use, including a change in route of administration;

(C) To change the strength;

(D) To make any other patented change regarding the drug, drug product, or any method of use.

(ii) If the applicant submits a supplement for one of the changes listed under paragraph (d)(2)(i) of this section and existing patents for which information has already been submitted to FDA claim the changed product, the applicant shall submit a certification with the supplement identifying the

patents that claim the changed product.

(iii) If the applicant submits a supplement for one of the changes listed under paragraph (d)(2)(i) of this section and no patents, including previously submitted patents, claim the changed product, it shall so certify.

(iv) The applicant shall comply with the requirements for amendment of formulation or composition and method of use patent information under paragraphs (c)(2)(ii) and (d)(3) of this section.

(3) *Patent information deadline.* If a patent is issued for a drug, drug product, or method of use after an application is approved, the applicant shall submit to FDA the required patent information within 30 days of the date of issuance of the patent.

(4) *Copies.* The applicant shall submit two copies of each submission of patent information, an archival copy and a copy for the chemistry, manufacturing, and controls section of the review copy, to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, Park Bldg., rm. 2–14, 12420 Parklawn Dr., Rockville, MD 20857. The applicant shall submit the patent information by letter separate from, but at the same time as, submission of the supplement.

(5) *Submission date.* Patent information shall be considered to be submitted to FDA as of the date the information is received by the Central Document Room.

(6) *Identification.* Each submission of patent information, except information submitted with an original application, and its mailing cover shall bear prominent identification as to its contents, i.e., ''Patent Information,'' or, if submitted after approval of an application, ''Time Sensitive Patent Information.''

(e) *Public disclosure of patent information.* FDA will publish in the list the patent number and expiration date of each patent that is required to be, and is, submitted to FDA by an applicant, and for each use patent, the approved indications or other conditions of use covered by a patent. FDA will publish such patent information upon approval of the application, or, if the patent information is submitted by the applicant after approval of an application as provided under paragraph (d)(2) of this section, as soon as possible after the submission to the agency of the patent information. Patent information submitted by the last working day of a month will be published in that month's supplement to the list. Patent information received by the agency between monthly publication of supplements to the list will be placed on public display in FDA's Freedom of Information Staff. A request for copies of the file shall be sent in writing to the Freedom of Information Staff (HFI–35), Food and Drug Administration, rm. 12A–16, 5600 Fishers Lane, Rockville, MD 20857.

(f) *Correction of patent information errors.* If any person disputes the accuracy or relevance of patent information submitted to the agency under this section and published by FDA in the list, or believes that an applicant has failed to submit required patent information, that person must first notify the agency in writing stating the grounds for disagreement. Such notification should be directed to the Drug Information Services Branch (HFD–84), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. The agency will then request of the applicable new drug application holder that the correctness of the patent information or omission of patent information be confirmed. Unless the application holder withdraws or amends its patent information in response to FDA's request, the agency will not change the patent information in the list. If the new drug application holder does not change the patent information submitted to FDA, a 505(b)(2) application or an abbreviated new drug application under section 505(j) of the act submitted for a drug that is claimed by a patent for which information has been submitted must, despite any disagreement as to the correctness of the patent information, contain an appropriate certification for each listed patent.

[59 FR 50363, Oct. 3, 1994]

§ 314.54 Procedure for submission of an application requiring investigations for approval of a new indication for, or other change from, a listed drug.

(a) The act does not permit approval of an abbreviated new drug application for a new indication, nor does it permit approval of other changes in a listed drug if investigations, other than bioavailability or bioequivalence studies, are essential to the approval of the change. Any person seeking approval of a drug product that represents a modification of a listed drug (e.g., a new indication or new dosage form) and for which investigations, other than bioavailability or bioequivalence studies, are essential to the approval of the changes may, except as provided in paragraph (b) of this section, submit a 505(b)(2) application. This application need contain only that information needed to support the modification(s) of the listed drug.

(1) The applicant shall submit a complete archival copy of the application that contains the following:

(i) The information required under § 314.50(a), (b), (c), (d)(1), (d)(3), (e), and (g), except that § 314.50(d)(1)(ii)(*c*) shall contain the proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product.

(ii) The information required under § 314.50 (d)(2), (d)(4) (if an anti-infective drug), (d)(5), (d)(6), and (f) as needed to support the safety and effectiveness of the drug product.

(iii) Identification of the listed drug for which FDA has made a finding of safety and effectiveness and on which finding the applicant relies in seeking approval of its proposed drug product by established name, if any, proprietary name, dosage form, strength, route of administration, name of listed drug's application holder, and listed drug's approved application number.

(iv) If the applicant is seeking approval only for a new indication and not for the indications approved for the listed drug on which the applicant relies, a certification so stating.

(v) Any patent information required under section 505(b)(1) of the act with respect to any patent which claims the drug for which approval is sought or a method of using such drug and to which a claim of patent infringement could reasonably be asserted if a person not licensed by the owner of the patent engaged in the manufacture, use, or sale of the drug product.

(vi) Any patent certification or statement required under section 505(b)(2) of the act with respect to any relevant patents that claim the listed drug or that claim any other drugs on which investigations relied on by the applicant for approval of the application were conducted, or that claim a use for the listed or other drug.

(vii) If the applicant believes the change for which it is seeking approval is entitled to a period of exclusivity, the information required under § 314.50(j).

(2) The applicant shall submit a review copy that contains the technical sections described in § 314.50(d)(1), except that § 314.50(d)(1)(ii)(*c*) shall contain the proposed or actual master production record, including a description of the equipment, to be used for the manufacture of a commercial lot of the drug product, and paragraph (d)(3), and the technical sections described in paragraphs (d)(2), (d)(4), (d)(5), (d)(6), and (f) when needed to support the modification. Each of the technical sections in the review copy is required to be separately bound with a copy of the information required under § 314.50 (a), (b), and (c) and a copy of the proposed labeling.

(3) The information required by § 314.50 (d)(2), (d)(4) (if an anti-infective drug), (d)(5), (d)(6), and (f) for the listed drug on which the applicant relies shall be satisfied by reference to the listed drug under paragraph (a)(1)(iii) of this section.

(4) The applicant shall submit a field copy of the application that contains the technical section described in § 314.50(d)(1), a copy of the information required under § 314.50(a) and (c), and certification that the field copy is a true copy of the technical section described in § 314.50(d)(1) contained in the archival and review copies of the application.

(b) An application may not be submitted under this section for a drug

product whose only difference from the reference listed drug is that:

(1) The extent to which its active ingredient(s) is absorbed or otherwise made available to the site of action is less than that of the reference listed drug; or

(2) The rate at which its active ingredient(s) is absorbed or otherwise made available to the site of action is unintentionally less than that of the reference listed drug.

[57 FR 17982, Apr. 28, 1992; 57 FR 61612, Dec. 28, 1992, as amended at 58 FR 47351, Sept. 8, 1993; 59 FR 50364, Oct. 3, 1994]

§ 314.60 Amendments to an unapproved application.

(a) Except as provided in paragraph (b) of this section, the applicant may submit an amendment to an application that is filed under § 314.100, but not yet approved. The submission of a major amendment (for example, an amendment that contains significant new data from a previously unreported study or detailed newnalyses of previously submitted data), whether on the applicant's own initiative or at the invitation of the agency, constitutes an agreement by the applicant under section 505(c) of the act to extend the date by which the agency is required to reach a decision on the application. Ordinarily, the agency will extend the review period for a major amendment but only for the time necessary to review the new information. However, the agency may not extend the review period more than 180 days. If the agency extends the review period for the application, the director of the division responsible for reviewing the application will notify the applicant of the length of the extension. The submission of an amendment that is not a major amendment will not extend the review period. An amendment that contains new clinical data from a previously unreported study shall contain a financial certification or disclosure statement or both as required by part 54 of this chapter, or FDA may refuse to accept any such amendment.

(b)(1) An unapproved application may not be amended if all of the following conditions apply:

(i) The unapproved application is for a drug for which a previous application has been approved and granted a period of exclusivity in accordance with section 505(c)(3)(D)(ii) of the act that has not expired;

(ii) The applicant seeks to amend the unapproved application to include a published report of an investigation that was conducted or sponsored by the applicant entitled to exclusivity for the drug;

(iii) The applicant has not obtained a right of reference to the investigation described in paragraph (b)(1)(ii) of this section; and

(iv) The report of the investigation described in paragraph (b)(1)(ii) of this section would be essential to the approval of the unapproved application.

(2) The submission of an amendment described in paragraph (b)(1) of this section will cause the unapproved application to be deemed to be withdrawn by the applicant under § 314.65 on the date of receipt by FDA of the amendment. The amendment will be considered a resubmission of the application, which may not be accepted except as provided in accordance with section 505(c)(3)(D)(ii) of the act.

(c) The applicant shall submit a field copy of each amendment to § 314.50(d)(1). The applicant, other than a foreign applicant, shall include in its submission of each such amendment to FDA a statement certifying that a field copy of the amendment has been sent to the applicant's home FDA district office.

[50 FR 7493, Feb. 22, 1985, as amended at 57 FR 17983, Apr. 28, 1992; 58 FR 47352, Sept. 8, 1993; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 314.60 was amended in paragraph (a) by adding a new sentence at the end of the paragraph, effective Feb. 2, 1999.

§ 314.65 Withdrawal by the applicant of an unapproved application.

An applicant may at any time withdraw an application that is not yet approved by notifying the Food and Drug Administration in writing. The agency will consider an applicant's failure to respond within 10 days to an approvable letter under § 314.110 or a not approvable letter under § 314.120 to be a request by the applicant to withdraw the application. A decision to withdraw the application is without prejudice to

refiling. The agency will retain the application and will provide a copy to the applicant on request under the fee schedule in § 20.42 of FDA's public information regulations.

§ 314.70 Supplements and other changes to an approved application.

(a) *Changes to an approved application.* The applicant shall notify FDA about each change in each condition established in an approved application beyond the variations already provided for in the application. The notice is required to describe the change fully. Depending on the type of change, the applicant shall notify FDA about it in a supplemental application under paragraph (b) or (c) of this section or by inclusion of the information in the annual report to the application under paragraph (d) of this section. Notwithstanding the requirements of paragraphs (b) and (c) of this section, an applicant shall make a change provided for in those paragraphs (for example, the deletion of an ingredient common to many drug products) in accordance with a guideline, notice, or regulation published in the FEDERAL REGISTER that provides for a less burdensome notification of the change (for example, by notification at the time a supplement is submitted or in the next annual report). Except for a supplemental application providing for a change in the labeling, the applicant, other than a foreign applicant, shall include in each supplemental application providing for a change under paragraph (b) or (c) of this section a statement certifying that a field copy of the supplement has been provided to the applicant's home FDA district office.

(b) *Supplements requiring FDA approval before the change is made.* An applicant shall submit a supplement, and obtain FDA approval of it, before making the changes listed below in the conditions in an approved application, unless the change is made to comply with an official compendium. An applicant may ask FDA to expedite its review of a supplement if a delay in making the change described in it would impose an extraordinary hardship on the applicant. Such a supplement and its mailing cover should be plainly marked: ''Supplement—Expedited Review Requested.''

(1) *Drug substance.* A change affecting the drug substance to accomplish any of the following:

(i) To relax the limits for a specification;

(ii) To establish a new regulatory analytical method;

(iii) To delete a specification or regulatory analytical method;

(iv) To change the synthesis of the drug substance, including a change in solvents and a change in the route of synthesis.

(v) To use a different facility or establishment to manufacture the drug substance, where: (*a*) the manufacturing process in the new facility or establishment differs materially from that in the former facility or establishment, or (*b*) the new facility or establishment has not received a satisfactory current good manufacturing practice (CGMP) inspection within the previous 2 years covering that manufacturing process.

(2) *Drug product.* A change affecting the drug product to accomplish any of the following:

(i) To add or delete an ingredient, or otherwise to change the composition of the drug product, other than deletion of an ingredient intended only to affect the color of the drug product;

(ii) To relax the limits for a specification;

(iii) To establish a new regulatory analytical method;

(iv) To delete a specification or regulatory analytical method;

(v) To change the method of manufacture of the drug product, including changing or relaxing an in-process control;

(vi) To use a different facility or establishment, including a different contract laboratory or labeler, to manufacture, process, or pack the drug product;

(vii) To change the container and closure system for the drug product (for example, glass to high density polyethylene (HDPE), or HDPE to polyvinyl chloride) or change a specification or regulatory analytical method for the container and closure system;

(viii) To change the size of the container, except for solid dosage forms,

without a change in the container and closure system.

(ix) To extend the expiration date of the drug product based on data obtained under a new or revised stability testing protocol that has not been approved in the application.

(x) To establish a new procedure for reprocessing a batch of the drug product that fails to meet specifications.

(xi) To add a code imprint by printing with ink on a solid oral dosage form drug product.

(xii) To add a code imprint by embossing, debossing, or engraving on a modified release solid oral dosage form drug product.

(3) *Labeling.* Any change in labeling, except one described in paragraph (c)(2) or (d) of this section.

(c) *Supplements for changes that may be made before FDA approval.* An applicant shall submit a supplement at the time the applicant makes any kind of change listed below in the conditions in an approved application, unless the change is made to comply with an official compendium. A supplement under this paragraph is required to give a full explanation of the basis for the change, identify the date on which the change is made, and, if the change concerns labeling, include 12 copies of final printed labeling. The applicant shall promptly revise all promotional labeling and drug advertising to make it consistent with any change in the labeling. The supplement and its mailing cover should be plainly marked: ''Special Supplement—Changes Being Effected.''

(1) Adds a new specification or test method or changes in the methods, facilities (except a change to a new facility), or controls to provide increased assurance that the drug will have the characteristics of identity, strength, quality, and purity which it purports or is represented to possess;

(2) Changes labeling to accomplish any of the following:

(i) To add or strengthen a contraindication, warning, precaution, or adverse reaction;

(ii) To add or strengthen a statement about drug abuse, dependence, or overdosage; or

(iii) To add or strengthen an instruction about dosage and administration that is intended to increase the safe use of the product.

(iv) To delete false, misleading, or unsupported indications for use or claims for effectiveness.

(3) To use a different facility or establishment to manufacture the drug substance, where: (i) The manufacturing process in the new facility or establishment does not differ materially from that in the former facility or establishment, and (ii) the new facility or establishment has received a satisfactory current good manufacturing practice (CGMP) inspection within the previous 2 years covering that manufacturing process.

(d) *Changes described in the annual report.* An applicant shall not submit a supplement to make any change in the conditions in an approved application, unless otherwise required under paragraph (b) or (c) of this section, but shall describe the change in the next annual report required under § 314.81. Some examples of changes that can be described in the annual report are the following:

(1) Any change made to comply with an official compendium.

(2) A change in the labeling concerning the description of the drug product or in the information about how the drug product is supplied, that does not involve a change in the dosage strength or dosage form.

(3) An editorial or similar minor change in labeling.

(4) The deletion of an ingredient intended only to affect the color of the drug product.

(5) An extension of the expiration date based upon full shelf-life data obtained from a protocol approved in the application.

(6) A change within the container and closure system for the drug product (for example, a change from one high density polyethylene (HDPE) to another HDPE), except a change in container size for nonsolid dosage forms, based upon a showing of equivalency to the approved system under a protocol approved in the application or published in an official compendium.

(7) The addition or deletion of an alternate analytical method.

(8) A change in the size of a container for a solid dosage form, without a

change from one container and closure system to another.

(9) The addition by embossing, debossing, or engraving of a code imprint to a solid oral dosage form drug product other than a modified release dosage form, or a minor change in an existing code imprint.

(e) *Patent information.* The applicant shall comply with the patent information requirements under section 505(c)(2) of the act.

(f) *Claimed exclusivity.* If an applicant claims exclusivity under § 314.108 upon approval of a supplemental application for a change to its previously approved drug product, the applicant shall include with its supplemental application the information required under § 314.50(j).

(g) *Exception.* An applicant proposing to make a change of a type described in paragraphs (a), (b)(1), (b)(2), (c)(1), (c)(3), (d)(1), and (d)(4) through (d)(9) of this section affecting a recombinant DNA-derived protein/polypeptide product or a complex or conjugate of a drug with a monoclonal antibody regulated under the Federal Food, Drug, and Cosmetic Act shall comply with the following:

(1) *Changes requiring supplement submission and approval prior to distribution of the product made using the change (major changes).* (i) A supplement shall be submitted for any change in the product, production process, quality controls, equipment, or facilities that has a substantial potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product.

(ii) These changes include, but are not limited to:

(A) Changes in the qualitative or quantitative formulation or other specifications as provided in the approved application or in the regulations;

(B) Changes requiring completion of an appropriate human study to demonstrate the equivalence of the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product;

(C) Changes in the virus or adventitious agent removal or inactivation method(s);

(D) Changes in the source material or cell line;

(E) Establishment of a new master cell bank or seed; and

(F) Changes which may affect product sterility assurance, such as changes in product or component sterilization method(s) or an addition, deletion, or substitution of steps in an aseptic processing operation.

(iii) The applicant must obtain approval of the supplement from FDA prior to distribution of the product made using the change. Except for submissions under paragraph (g)(4) of this section, the following shall be contained in the supplement:

(A) A detailed description of the proposed change;

(B) The product(s) involved;

(C) The manufacturing site(s) or area(s) affected;

(D) A description of the methods used and studies performed to evaluate the effect of the change on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product;

(E) The data derived from such studies;

(F) Relevant validation protocols and data; and

(G) A reference list of relevant standard operating procedures (SOP's).

(2) *Changes requiring supplement submission at least 30 days prior to distribution of the product made using the change.* (i) A supplement shall be submitted for any change in the product, production process, quality controls, equipment, or facilities that has a moderate potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. The supplement shall be labeled ''Supplement—Changes Being Effected in 30 Days'' or, if applicable under paragraph (g)(2)(v) of this section, ''Supplement—Changes Being Effected.''

(ii) These changes include, but are not limited to:

(A) Change in the site of testing from one facility to another;

(B) An increase or decrease in production scale during finishing steps that involves new or different equipment; and

(C) Replacement of equipment with that of similar, but not identical, design and operating principle that does not affect the process methodology or process operating parameters.

(iii) Pending approval of the supplement by FDA, and except as provided in paragraph (g)(2)(v) of this section, distribution of the product made using the change may begin not less than 30 days after receipt of the supplement by FDA. The information listed in paragraph (g)(1)(iii)(A) through (g)(1)(iii)(G) of this section shall be contained in the supplement.

(iv) If within 30 days following FDA's receipt of the supplement, FDA informs the applicant that either:

(A) The change requires approval prior to distribution of the product in accordance with paragraph (g)(1) of this section; or

(B) Any of the information required under paragraph (g)(2)(iii) of this section is missing; the applicant shall not distribute the product made using the change until FDA determines that compliance with this section is achieved.

(v) In certain circumstances, FDA may determine that, based on experience with a particular type of change, the supplement for such change is usually complete and provides the proper information, and on particular assurances that the proposed change has been appropriately submitted, the product made using the change may be distributed immediately upon receipt of the supplement by FDA. These circumstances may include substantial similarity with a type of change regularly involving a ''Supplement—Changes Being Effected'' supplement, or a situation in which the applicant presents evidence that the proposed change has been validated in accordance with an approved protocol for such change under paragraph (g)(4) of this section.

(3) *Changes to be described in an annual report (minor changes).* (i) Changes in the product, production process, quality controls, equipment, or facilities that have a minimal potential to have an adverse effect on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product shall be documented by the applicant in the next annual report in accordance with § 314.81(b)(2)(iv).

(ii) These changes include, but are not limited to:

(A) Any change made to comply with an official compendium that is consistent with FDA requirements;

(B) The deletion of an ingredient intended only to affect the color of the product;

(C) An extension of an expiration date based upon full shelf life data obtained from a protocol approved in the application;

(D) A change within the container and closure system for solid dosage forms, based upon a showing of equivalency to the approved system under a protocol approved in the application or published in an official compendium;

(E) A change in the size of a container for a solid dosage form, without a change from one container and closure system to another;

(F) The addition by embossing, debossing, or engraving of a code imprint to a solid dosage form drug product other than a modified release dosage form, or a minor change in an existing code imprint; and

(G) The addition or deletion of an alternate analytical method.

(4) An applicant may submit one or more protocols describing the specific tests and validation studies and acceptable limits to be achieved to demonstrate the lack of adverse effect for specified types of manufacturing changes on the identity, strength, quality, purity, or potency of the product as they may relate to the safety or effectiveness of the product. Any such protocols, or change to a protocol, shall be submitted as a supplement requiring approval from FDA prior to distribution of the product which, if approved, may justify a reduced reporting category for the particular change because the use of the protocol for that

type of change reduces the potential risk of an adverse effect.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 57 FR 17983, Apr. 28, 1992; 58 FR 47352, Sept. 8, 1993; 58 FR 47959, Sept. 13, 1993; 59 FR 50364, Oct. 3, 1994; 62 FR 39900, July 24, 1997]

§ 314.71 Procedures for submission of a supplement to an approved application.

(a) Only the applicant may submit a supplement to an application.

(b) All procedures and actions that apply to an application under § 314.50 also apply to supplements, except that the information required in the supplement is limited to that needed to support the change. A supplement is required to contain an archival copy and a review copy that include an application form and appropriate technical sections, samples, and labeling; except that a supplement for a change other than a change in labeling is required also to contain a field copy.

(c) All procedures and actions that apply to applications under this part, including actions by applicants and the Food and Drug Administration, also apply to supplements.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 58 FR 47352, Sept. 8, 1993]

§ 314.72 Change in ownership of an application.

(a) An applicant may transfer ownership of its application. At the time of transfer the new and former owners are required to submit information to the Food and Drug Administration as follows:

(1) The former owner shall submit a letter or other document that states that all rights to the application have been transferred to the new owner.

(2) The new owner shall submit an application form signed by the new owner and a letter or other document containing the following:

(i) The new owner's commitment to agreements, promises, and conditions made by the former owner and contained in the application;

(ii) The date that the change in ownership is effective; and

(iii) Either a statement that the new owner has a complete copy of the approved application, including supplements and records that are required to be kept under § 314.81, or a request for a copy of the application from FDA's files. FDA will provide a copy of the application to the new owner under the fee schedule in § 20.42 of FDA's public information regulations.

(b) The new owner shall advise FDA about any change in the conditions in the approved application under § 314.70, except the new owner may advise FDA in the next annual report about a change in the drug product's label or labeling to change the product's brand or the name of its manufacturer, packer, or distributor.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985]

§ 314.80 Postmarketing reporting of adverse drug experiences.

(a) *Definitions.* The following definitions of terms apply to this section:–

Adverse drug experience. Any adverse event associated with the use of a drug in humans, whether or not considered drug related, including the following: An adverse event occurring in the course of the use of a drug product in professional practice; an adverse event occurring from drug overdose whether accidental or intentional; an adverse event occurring from drug abuse; an adverse event occurring from drug withdrawal; and any failure of expected pharmacological action.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient, in the view of the initial reporter, at *immediate* risk of death from the adverse drug experience as it occurred, i.e., it does not include an adverse drug experience that, had it

occurred in a more severe form, might have caused death.

Serious adverse drug experience. Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience. Any adverse drug experience that is not listed in the current labeling for the drug product. This includes events that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differ from the event because of greater severity or specificity. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the labeling only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the labeling only listed cerebral vascular accidents. ''Unexpected,'' as used in this definition, refers to an adverse drug experience that has not been previously observed (i.e., included in the labeling) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) *Review of adverse drug experiences.* Each applicant having an approved application under § 314.50 or, in the case of a 505(b)(2) application, an effective approved application, shall promptly review all adverse drug experience information obtained or otherwise received by the applicant from any source, foreign or domestic, including information derived from commercial marketing experience, postmarketing clinical investigations, postmarketing epidemiological/surveillance studies, reports in the scientific literature, and unpublished scientific papers. Applicants are not required to resubmit to FDA adverse drug experience reports forwarded to the applicant by FDA; however, applicants must submit all followup information on such reports to FDA. Any person subject to the reporting requirements under paragraph (c) of this section shall also develop written procedures for the surveillance, receipt, evaluation, and reporting of postmarketing adverse drug experiences to FDA.

(c) *Reporting requirements.* The applicant shall report to FDA adverse drug experience information, as described in this section. The applicant shall submit two copies of each report described in this section to the Central Document Room, 12229 Wilkins Ave., Rockville, MD 20852. FDA may waive the requirement for the second copy in appropriate instances.

(1)(i) *Postmarketing 15-day ''Alert reports''.* The applicant shall report each adverse drug experience that is both serious and unexpected, whether foreign or domestic, as soon as possible but in no case later than 15 calendar days of initial receipt of the information by the applicant.

(ii) *Postmarketing 15-day ''Alert reports''—followup.* The applicant shall promptly investigate all adverse drug experiences that are the subject of these postmarketing 15-day Alert reports and shall submit followup reports within 15 calendar days of receipt of new information or as requested by FDA. If additional information is not obtainable, records should be maintained of the unsuccessful steps taken to seek additional information. Postmarketing 15-day Alert reports and followups to them shall be submitted under separate cover.

(iii) *Submission of reports.* The requirements of paragraphs (c)(1)(i) and (c)(1)(ii) of this section, concerning the submission of postmarketing 15-day

Alert reports, shall also apply to any person other than the applicant (nonapplicant) whose name appears on the label of an approved drug product as a manufacturer, packer, or distributor. To avoid unnecessary duplication in the submission to FDA of reports required by paragraphs (c)(1)(i) and (c)(1)(ii) of this section, obligations of a nonapplicant may be met by submission of all reports of serious adverse drug experiences to the applicant. If a nonapplicant elects to submit adverse drug experience reports to the applicant rather than to FDA, the nonapplicant shall submit each report to the applicant within 5 calendar days of receipt of the report by the nonapplicant, and the applicant shall then comply with the requirements of this section. Under this circumstance, the nonapplicant shall maintain a record of this action which shall include:

(A) A copy of each adverse drug experience report;

(B) The date the report was received by the nonapplicant;

(C) The date the report was submitted to the applicant; and

(D) The name and address of the applicant.

(iv) *Report identification.* Each report submitted under this paragraph shall bear prominent identification as to its contents, i.e., ''15-day Alert report,'' or ''15-day Alert report-followup.''

(2) *Periodic adverse drug experience reports.* (i) The applicant shall report each adverse drug experience not reported under paragraph (c)(1)(i) of this section at quarterly intervals, for 3 years from the date of approval of the application, and then at annual intervals. The applicant shall submit each quarterly report within 30 days of the close of the quarter (the first quarter beginning on the date of approval of the application) and each annual report within 60 days of the anniversary date of approval of the application. Upon written notice, FDA may extend or reestablish the requirement that an applicant submit quarterly reports, or require that the applicant submit reports under this section at different times than those stated. For example, the agency may reestablish a quarterly reporting requirement following the approval of a major supplement. Followup information to adverse drug experiences submitted in a periodic report may be submitted in the next periodic report.

(ii) Each periodic report is required to contain: (*a*) a narrative summary and analysis of the information in the report and an analysis of the 15-day Alert reports submitted during the reporting interval (all 15-day Alert reports being appropriately referenced by the applicant's patient identification number, adverse reaction term(s), and date of submission to FDA); (*b*) a FDA Form 3500A (Adverse Reaction Report) for each adverse drug experience not reported under paragraph (c)(1)(i) of this section (with an index consisting of a line listing of the applicant's patient identification number and adverse reaction term(s)); and (*c*) a history of actions taken since the last report because of adverse drug experiences (for example, labeling changes or studies initiated).

(iii) Periodic reporting, except for information regarding 15-day Alert reports, does not apply to adverse drug experience information obtained from postmarketing studies (whether or not conducted under an investigational new drug application), from reports in the scientific literature, and from foreign marketing experience.

(d) *Scientific literature.* (1) A 15-day Alert report based on information from the scientific literature is required to be accompanied by a copy of the published article. The 15-day reporting requirements in paragraph (c)(1)(i) of this section (i.e., serious, unexpected adverse drug experiences) apply only to reports found in scientific and medical journals either as case reports or as the result of a formal clinical trial.

(2) As with all reports submitted under paragraph (c)(1)(i) of this section, reports based on the scientific literature shall be submitted on FDA Form 3500A or comparable format as prescribed by paragraph (f) of this section. In cases where the applicant believes that preparing the FDA Form 3500A constitutes an undue hardship, the applicant may arrange with the Division of Pharmacovigilance and Epidemiology for an acceptable alternative reporting format.

(e) *Postmarketing studies.* (1) An applicant is not required to submit a 15-day Alert report under paragraph (c) of this section for an adverse drug experience obtained from a postmarketing study (whether or not conducted under an investigational new drug application) unless the applicant concludes that there is a reasonable possibility that the drug caused the adverse experience.

(2) The applicant shall separate and clearly mark reports of adverse drug experiences that occur during a postmarketing study as being distinct from those experiences that are being reported spontaneously to the applicant.

(f) *Reporting FDA Form 3500A.* (1) Except as provided in paragraph (f)(3) of this section, the applicant shall complete FDA Form 3500A for each report of an adverse drug experience (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form).

(2) Each completed FDA Form 3500A should refer only to an individual patient or a single attached publication.

(3) Instead of using FDA Form 3500A, an applicant may use a computer-generated FDA Form 3500A or other alternative format (e.g., a computer-generated tape or tabular listing) provided that: (i) The content of the alternative format is equivalent in all elements of information to those specified in FDA Form 3500A; and (ii) The format is agreed to in advance by MedWatch: The FDA Medical Products Reporting Program.

(4) Ten copies or fewer of FDA Form 3500A and/or a copy of the instructions for completing the form may be obtained from the Division of Pharmacovigilance and Epidemiology (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. More than 10 copies of the form may be obtained by writing to the Consolidated Forms and Publications Distribution Center, Washington Commerce Center, 3222 Hubbard Rd., Landover, MD 20785.

(g) *Multiple reports.* An applicant should not include in reports under this section any adverse drug experiences that occurred in clinical trials if they were previously submitted as part of the approved application. If a report applies to a drug for which an applicant holds more than one approved application, the applicant should submit the report to the application that was first approved. If a report refers to more than one drug marketed by an applicant, the applicant should submit the report to the application for the drug listed first in the report.

(h) *Patient privacy.* An applicant should not include in reports under this section the names and addresses of individual patients; instead, the applicant should assign a unique code number to each report, preferably not more than eight characters in length. The applicant should include the name of the reporter from whom the information was received. Names of patients, health care professionals, hospitals, and geographical identifiers in adverse drug experience reports are not releasable to the public under FDA's public information regulations in part 20.

(i) *Recordkeeping.* The applicant shall maintain for a period of 10 years records of all adverse drug experiences known to the applicant, including raw data and any correspondence relating to adverse drug experiences.

(j) *Withdrawal of approval.* If an applicant fails to establish and maintain records and make reports required under this section, FDA may withdraw approval of the application and, thus, prohibit continued marketing of the drug product that is the subject of the application.

(k) *Disclaimer.* A report or information submitted by an applicant under this section (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the applicant or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse effect. An applicant need not admit, and may deny, that the report or information submitted under this section constitutes an admission that the drug caused or contributed to an adverse effect. For purposes of this

provision, the term ''applicant'' also includes any person reporting under paragraph (c)(1)(iii) of this section.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 51 FR 24481, July 3, 1986; 52 FR 37936, Oct. 13, 1987; 55 FR 11580, Mar. 29, 1990; 57 FR 17983, Apr. 28, 1992; 62 FR 34168, June 25, 1997; 62 FR 52251, Oct. 7, 1997; 63 FR 14611, Mar. 26, 1998]

EFFECTIVE DATE NOTE: At 62 FR 52251, Oct. 7, 1997, § 314.80 was amended by revising paragraphs (a), (c)(1), (f)(1), (f)(3)(ii), and (f)(4) and the introductory text of paragraph (c); by adding two new sentences at the end of paragraph (b); by removing in paragraph (d)(2) the words ''Epidemiology and Surveillance'' and adding in their place the words ''Pharmacovigilance and Epidemiology''; by removing in paragraphs (c)(2)(ii)(b), (d)(2), (f)(2), and (f)(3) and in the heading for paragraph (f) the words ''Form FDA–1639'' or ''FDA–1639'' and adding in their place the words ''FDA Form 3500A''; and by removing paragraph (j) and redesignating paragraphs (k) and (l) as paragraphs (j) and (k), respectively, effective Apr. 6, 1998. For the convenience of the user, the superseded text is set forth as follows:

§ 314.80 Postmarketing reporting of adverse drug experiences.

(a) *Definitions.* The following definitions of terms apply to this section:

Adverse drug experience means any adverse event associated with the use of a drug in humans, whether or not considered drug related, including the following: an adverse event occurring in the course of the use of a drug product in professional practice; an adverse event occurring from drug overdose, whether accidental or intentional; an adverse event occurring from drug abuse; an adverse event occurring from drug withdrawal; and any failure of expected pharmacological action.

Serious means an adverse drug experience that is fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, or is a congenital anomaly, cancer, or overdose.

Unexpected means an adverse drug experience that is not listed in the current labeling for the drug and includes an event that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differs from the event because of greater severity or specificity. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the labeling only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the labeling only listed cerebral vascular accidents.

* * * * *

(c) *Reporting requirements.* The applicant shall report to FDA adverse drug experience information, as described in this section. The applicant shall submit two copies of each report described in this section to the Central Document Room, Park Bldg., Rm. 214, 12420 Parklawn Dr., Rockville, MD 20852. FDA may waive the requirement for the second copy in appropriate instances.

(1) Fifteen-day ''Alert reports.'' (i) The applicant shall report each adverse drug experience that is both serious and unexpected, regardless of source, as soon as possible but in any case within 15 working days of initial receipt of the information. These reports are required to be submitted on Form FDA–1639 (Adverse Reaction Report). The applicant shall promptly investigate all adverse drug experiences that are the subject of these 15-day Alert reports and shall submit followup reports within 15 working days of receipt of new information or as requested by FDA. If additional information is not obtainable, a followup report may be required that describes briefly the steps taken to seek additional information and the reasons why it could not be obtained. These 15-day Alert reports and followups to them are required to be submitted under separate cover and may not be included, except for summary or tabular purposes, in a periodic report.

(ii) The requirements of paragraph (c)(1)(i) of this section, concerning the submission of 15-day Alert reports, shall also apply to any person (other than the applicant) whose name appears on the label of an approved drug product as a manufacturer, packer, or distributor. However, to avoid unnecessary duplication in the submission to FDA of, and followup to, reports required by paragraph (c)(1)(i) of this section, obligations of a nonapplicant may be met by submission of all reports of serious adverse drug experiences to the applicant. If a nonapplicant elects to submit adverse drug experience reports to the applicant rather than to FDA, it shall submit each report to the applicant within 3 working days of its receipt by the nonapplicant, and the applicant shall then comply with the requirements of this section. Under this circumstance, the nonapplicant shall maintain a record of this action which shall include:

(*a*) A copy of the drug experience report.

(*b*) Date the report was received by the nonapplicant.

(*c*) Date the report was submitted to the applicant.

(*d*) Name and address of the applicant.

(iii) Each report submitted under this paragraph shall bear prominent identification as to its contents, i.e., ''15-day Alert report'' or ''15-day Alert report—followup.''

* * * * *

(f) *Reporting Form FDA–1639.* (1) Except as provided in paragraph (f)(3) of this section, the applicant shall complete a Form FDA–1639 (Adverse Reaction Report) for each report of an adverse drug experience.

* * * * *

(3) * * *

(ii) the format is agreed to in advance by the Division of Epidemiology and Surveillance (HFD–730).

(4) Single copies of Form FDA–1639 may be obtained from the Division of Epidemiology and Surveillance (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Supplies of Form FDA–1639 may be obtained from the PHS Forms and Publications Distribution Center, 12100 Parklawn Dr., Rockville, MD 20857.

* * * * *

(j) *Guideline.* FDA has prepared under § 10.90(b) a guideline for the submission of reports of adverse drug experiences and suggested followup investigation of reports.

* * * * *

2. At 63 FR 14611, Mar. 26, 1998, § 314.80 was amended by correcting the reference to ''paragraph (c)(1)(ii)'' in the last sentence in newly redesignated paragraph (k) to read ''paragraph (c)(1)(iii)'', effective Apr. 6, 1998.

§ 314.81 Other postmarketing reports.

(a) *Applicability.* Each applicant shall make the reports for each of its approved applications and abbreviated applications required under this section and sections 505(k) and 507(g) of the act.

(b) *Reporting requirements.* The applicant shall submit to the Food and Drug Administration at the specified times two copies of the following reports:

(1) *NDA—Field alert report.* The applicant shall submit information of the following kinds about distributed drug products and articles to the FDA district office that is responsible for the facility involved within 3 working days of receipt by the applicant. The information may be provided by telephone or other rapid communication means, with prompt written followup. The report and its mailing cover should be plainly marked: ''NDA—Field Alert Report.''

(i) Information concerning any incident that causes the drug product or its labeling to be mistaken for, or applied to, another article.

(ii) Information concerning any bacteriological contn, or any significant chemical, physical, or other change or deterioration in the distributed drug product, or any failure of one or more distributed batches of the drug product to meet the specifications established for it in the application.

(2) *Annual report.* The applicant shall submit the following information in the order listed each year within 60 days of the anniversary date of approval of the application. The applicant shall submit the report to the FDA division responsible for reviewing the application. Each annual report is required to be accompanied by a completed transmittal Form FDA–2252 (Transmittal of Periodic Reports for Drugs for Human Use) which may be obtained from the PHS Forms and Publications Distribution Center, 12100 Parklawn Dr., Rockville, MD 20857, and is required to include all the information required under this section that the applicant received or otherwise obtained during the annual reporting interval which ends on the anniversary date. The report is required to contain the following:

(i) *Summary.* A brief summary of significant new information from the previous year that might affect the safety, effectiveness, or labeling of the drug product. The report is also required to contain a brief description of actions the applicant has taken or intends to take as a result of this new information, for example, submit a labeling supplement, add a warning to the labeling, or initiate a new study.

(ii) *Distribution data.* Information about the quantity of the drug product distributed under the approved application, including that distributed to distributors. The information is required to include the National Drug Code (NDC) number, the total number of dosage units of each strength or potency distributed (e.g., 100,000/5 milligram tablets, 50,000/10 milliliter vials),

and the quantities distributed for domestic use and the quantities distributed for foreign use. Disclosure of financial or pricing data is not required.

(iii) *Labeling.* Currently used professional labeling, patient brochures or package inserts (if any), a representative sample of the package labels, and a summary of any changes in labeling that have been made since the last report listed by date in the order in which they were implemented, or if no changes, a statement of that fact.

(iv) *Chemistry, manufacturing, and controls changes.* (*a*) Reports of experiences, investigations, studies, or tests involving chemical or physical properties, or any other properties of the drug (such as the drug's behavior or properties in relation to microorganisms, including both the effects of the drug on microorganisms and the effects of microorganisms on the drug). These reports are only required for new information that may affect FDA's previous conclusions about the safety or effectiveness of the drug product.

(*b*) A full description of the manufacturing and controls changes not requiring a supplemental application under § 314.70 (b) and (c), listed by date in the order in which they were implemented.

(v) *Nonclinical laboratory studies.* Copies of unpublished reports and summaries of published reports of new toxicological findings in animal studies and in vitro studies (e.g., mutagenicity) conducted by, or otherwise obtained by, the applicant concerning the ingredients in the drug product. The applicant shall submit a copy of a published report if requested by FDA.

(vi) *Clinical data.* (*a*) Published clinical trials of the drug (or abstracts of them), including clinical trials on safety and effectiveness; clinical trials on new uses; biopharmaceutic, pharmacokinetic, and clinical pharmacology studies; and reports of clinical experience pertinent to safety (for example, epidemiologic studies or analyses of experience in a monitored series of patients) conducted by or otherwise obtained by the applicant. Review articles, papers describing the use of the drug product in medical practice, papers and abstracts in which the drug is used as a research tool, promotional articles, press clippings, and papers that do not contain tabulations or summaries of original data should not be reported.

(*b*) Summaries of completed unpublished clinical trials, or prepublication manuscripts if available, conducted by, or otherwise obtained by, the applicant. Supporting information should not be reported. (A study is considered completed 1 year after it is concluded.)

(vii) *Status reports.* A statement on the current status of any postmarketing studies performed by, or on behalf of, the applicant. To facilitate communications between FDA and the applicant, the report may, at the applicant's discretion, also contain a list of any open regulatory business with FDA concerning the drug product subject to the application.

(3) *Other reporting*—(i) *Advertisements and promotional labeling.* The applicant shall submit specimens of mailing pieces and any other labeling or advertising devised for promotion of the drug product at the time of initial dissemination of the labeling and at the time of initial publication of the advertisement for a prescription drug product. Mailing pieces and labeling that are designed to contain samples of a drug product are required to be complete, except the sample of the drug product may be omitted. Each submission is required to be accompanied by a completed transmittal Form FDA–2253 (Transmittal of Advertisements and Promotional Labeling for Drugs for Human Use) and is required to include a copy of the product's current professional labeling. Form FDA–2253 may be obtained from the PHS Forms and Publications Distribution Center, 12100 Parklawn Dr., Rockville, MD 20857.

(ii) *Special reports.* Upon written request the agency may require that the applicant submit the reports under this section at different times than those stated.

(iii) *Withdrawal of approved drug product from sale.* (*a*) The applicant shall submit on Form FDA 2657 (Drug Product Listing), within 15 working days of the withdrawal from sale of a drug product, the following information:

(*1*) The National Drug Code (NDC) number.

(*2*) The identity of the drug product by established name and by proprietary name.

(*3*) The new drug application or abbreviated application number.

(*4*) The date of withdrawal from sale. It is requested but not required that the reason for withdrawal of the drug product from sale be included with the information.

(*b*) The applicant shall submit each Form FDA–2657 to the Drug Listing Branch (HFD–334), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(*c*) Reporting under paragraph (b)(3)(iii) of this section constitutes compliance with the requirements under § 207.30(a) of this chapter to report ''at the discretion of the registrant when the change occurs.''

(c) *General requirements*—(1) *Multiple applications.* For all reports required by this section, the applicant shall submit the information common to more than one application only to the application first approved, and shall not report separately on each application. The submission is required to identify all the applications to which the report applies.

(2) *Patient identification.* Applicants should not include in reports under this section the names and addresses of individual patients; instead, the applicant should code the patient names whenever possible and retain the code in the applicant's files. The applicant shall maintain sufficient patient identification information to permit FDA, by using that information alone or along with records maintained by the investigator of a study, to identify the name and address of individual patients; this will ordinarily occur only when the agency needs to investigate the reports further or when there is reason to believe that the reports do not represent actual results obtained.

(d) *Withdrawal of approval.* If an applicant fails to make reports required under this section, FDA may withdraw approval of the application and, thus, prohibit continued marketing of the drug product that is the subject of the application.

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[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11580, Mar. 29, 1990; 57 FR 17983, Apr. 28, 1992]

§ 314.90 Waivers.

(a) An applicant may ask the Food and Drug Administration to waive under this section any requirement that applies to the applicant under §§ 314.50 through 314.81. An applicant may ask FDA to waive under § 314.126(c) any criteria of an adequate and well-controlled study described in § 314.126(b). A waiver request under this section is required to be submitted with supporting documentation in an application, or in an amendment or supplement to an application. The waiver request is required to contain one of the following:

(1) An explanation why the applicant's compliance with the requirement is unnecessary or cannot be achieved;

(2) A description of an alternative submission that satisfies the purpose of the requirement; or

(3) Other information justifying a waiver.

(b) FDA may grant a waiver if it finds one of the following:

(1) The applicant's compliance with the requirement is unnecessary for the agency to evaluate the application or compliance cannot be achieved;

(2) The applicant's alternative submission satisfies the requirement; or

(3) The applicant's submission otherwise justifies a waiver.

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[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985]

Subpart C—Abbreviated Applications

SOURCE: 57 FR 17983, Apr. 28, 1992, unless otherwise noted.

§ 314.92 Drug products for which abbreviated applications may be submitted.

(a) Abbreviated applications are suitable for the following drug products within the limits set forth under § 314.93:

(1) Drug products that are the same as a listed drug. A ''listed drug'' is defined in § 314.3. For determining the suitability of an abbreviated new drug application, the term ''same as'' means identical in active ingredient(s), dosage form, strength, route of administration, and conditions of use, except that conditions of use for which approval cannot be granted because of exclusivity or an existing patent may be omitted. If a listed drug has been voluntarily withdrawn from or not offered for sale by its manufacturer, a person who wishes to submit an abbreviated new drug application for the drug shall comply with § 314.122.

(2) Drug products that are duplicates of, or that meet the monograph for, an antibiotic drug for which FDA has approved an application.

(3) Drug products that have been declared suitable for an abbreviated new drug application submission by FDA through the petition procedures set forth under § 10.30 of this chapter and § 314.93.

(b) FDA will publish in the list listed drugs for which abbreviated applications may be submitted. The list is available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402, 202–783–3238.

§ 314.93 Petition to request a change from a listed drug.

(a) The only changes from a listed drug for which the agency will accept a petition under this section are those changes described in paragraph (b) of this section. Petitions to submit abbreviated new drug applications for other changes from a listed drug will not be approved.

(b) A person who wants to submit an abbreviated new drug application for a drug product which is not identical to a listed drug in route of administration, dosage form, and strength, or in which one active ingredient is substituted for one of the active ingredients in a listed combination drug, must first obtain permission from FDA to submit such an abbreviated application.

(c) To obtain permission to submit an abbreviated new drug application for a change described in paragraph (b) of this section, a person must submit and obtain approval of a petition requesting the change. A person seeking permission to request such a change from a reference listed drug shall submit a petition in accordance with §10.20 of this chapter and in the format specified in § 10.30 of this chapter. The petition shall contain the information specified in § 10.30 of this chapter and any additional information required by this section. If any provision of § 10.20 or § 10.30 of this chapter is inconsistent with any provision of this section, the provisions of this section apply.

(d) The petitioner shall identify a listed drug and include a copy of the proposed labeling for the drug product that is the subject of the petition and a copy of the approved labeling for the listed drug. The petitioner may, under limited circumstances, identify more than one listed drug, for example, when the proposed drug product is a combination product that differs from the combination reference listed drug with regard to an active ingredient, and the different active ingredient is an active ingredient of a listed drug. The petitioner shall also include information to show that:

(1) The active ingredients of the proposed drug product are of the same pharmacological or therapeutic class as those of the reference listed drug.

(2) The drug product can be expected to have the same therapeutic effect as the reference listed drug when administered to patients for each condition of use in the reference listed drug's labeling for which the applicant seeks approval.

(3) If the proposed drug product is a combination product with one different active ingredient, including a different ester or salt, from the reference listed drug, that the different active ingredient has previously been approved in a listed drug or is a drug that does not meet the definition of ''new drug'' in section 201(b) of the act.

(e) No later than 90 days after the date a petition that is permitted under paragraph (a) of this section is submitted, FDA will approve or disapprove the petition.

(1) FDA will approve a petition properly submited under this section unless it finds that:

(i) Investigations must be conducted to show the safety and effectiveness of the drug product or of any of its active ingredients, its route of administration, dosage form, or strength which differs from the reference listed drug; or

(ii) For a petition that seeks to change an active ingredient, the drug product that is the subject of the petition is not a combination drug; or

(iii) For a combination drug product that is the subject of the petition and has an active ingredient different from the reference listed drug:

(A) The drug product may not be adequately evaluated for approval as safe and effective on the basis of the information required to be submitted under § 314.94; or

(B) The petition does not contain information to show that the different active ingredient of the drug product is of the same pharmacological or therapeutic class as the ingredient of the reference listed drug that is to be changed and that the drug product can be expected to have the same therapeutic effect as the reference listed drug when administered to patients for each condition of use in the listed drug's labeling for which the applicant seeks approval; or

(C) The different active ingredient is not an active ingredient in a listed drug or a drug that meets the requirements of section 201(p) of the act; or

(D) The remaining active ingredients are not identical to those of the listed combination drug; or

(iv) Any of the proposed changes from the listed drug would jeopardize the safe or effective use of the product so as to necessitate significant labeling changes to address the newly introduced safety or effectiveness problem; or

(v) FDA has determined that the reference listed drug has been withdrawn from sale for safety or effectiveness reasons under § 314.161, or the reference listed drug has been voluntarily withdrawn from sale and the agency has not determined whether the withdrawal is for safety or effectiveness reasons.

(2) For purposes of this paragraph, ''investigations must be conducted'' means that information derived from animal or clinical studies is necessary to show that the drug product is safe or effective. Such information may be contained in published or unpublished reports.

(3) If FDA approves a petition submitted under this section, the agency's response may describe what additional information, if any, will be required to support an abbreviated new drug application for the drug product. FDA may, at any time during the course of its review of an abbreviated new drug application, request additional information required to evaluate the change approved under the petition.

(f) FDA may withdraw approval of a petition if the agency receives any information demonstrating that the petition no longer satisfies the conditions under paragraph (e) of this section.

§ 314.94 Content and format of an abbreviated application.

Abbreviated applications are required to be submitted in the form and contain the information required under this section. Three copies of the application are required, an archival copy, a review copy, and a field copy. FDA will maintain guidelines on the format and content of applications to assist applicants in their preparation.

(a) *Abbreviated new drug applications.* Except as provided in paragraph (b) of this section, the applicant shall submit a complete archival copy of the abbreviated new drug application that includes the following:

(1) *Application form.* The applicant shall submit a completed and signed application form that contains the information described under § 314.50(a)(1), (a)(3), (a)(4), and (a)(5). The applicant shall state whether the submission is an abbreviated application under this section or a supplement to an abbreviated application under § 314.97.

(2) *Table of contents.* the archival copy of the abbreviated new drug application is required to contain a table of

contents that shows the volume number and page number of the contents of the submission.

(3) *Basis for abbreviated new drug application submission.* An abbreviated new drug application must refer to a listed drug. Ordinarily, that listed drug will be the drug product selected by the agency as the reference standard for conducting bioequivalence testing. The application shall contain:

(i) The name of the reference listed drug, including its dosage form and strength. For an abbreviated new drug application based on an approverd petition under §10.30 of this chapter or §314.93, the reference listed drug must be the same as the listed drug approved in the petition.

(ii) A statement as to whether, according to the information published in the list, the reference listed drug is entitled to a period of marketing exclusivity under section 505(j)(4)(D) of the act.

(iii) For an abbreviated new drug application based on an approved petition under § 10.30 of this chapter or § 314.93, a reference to FDA-assigned docket number for the petition and a copy of FDA's correspondence approving the petition.

(4) *Conditions of use.* (i) A statement that the conditions of use prescribed, recommended, or suggested in the labeling proposed for the drug product have been previously approved for the reference listed drug.

(ii) A reference to the applicant's annotated proposed labeling and to the currently approved labeling for the reference listed drug provided under paragraph (a)(8) of this section.

(5) *Active ingredients.* (i) For a single-active-ingredient drug product, information to show that the active ingredient is the same as that of the reference single-active-ingredient listed drug, as follows:

(A) A statement that the active ingredient of the proposed drug product is the same as that of the reference listed drug.

(B) A reference to the applicant's annotated proposed labeling and to the currently approved labeling for the reference listed drug provided under paragraph (a)(8) of this section.

(ii) For a combination drug product, information to show that the active ingredients are the same as those of the reference listed drug except for any different active ingredient that has been the subject of an approved petition, as follows:

(A) A statement that the active ingredients of the proposed drug product are the same as those of the reference listed drug, or if one of the active ingredients differs from one of the active ingredients of the reference listed drug and the abbreviated application is submitted under the approval of a petition under § 314.93 to vary such active ingredient, information to show that the other active ingredients of the drug product are the same as the other active ingredients of the reference listed drug, information to show that the different active ingredient is an active ingredient of another listed drug or of a drug that does not meet the definition of ''new drug'' in section 201(p) of the act, and such other information about the different active ingredient that FDA may require.

(B) A reference to the applicant's annotated proposed labeling and to the currently approved labeling for the reference listed drug provided under paragraph (a)(8) of this section.

(6) *Route of administration, dosage form, and strength.* (i) Information to show that the route of administration, dosage form, and strength of the drug product are the same as those of the reference listed drug except for any differences that have been the subject of an approved petition, as follows:

(A) A statement that the route of administration, dosage form, and strength of the proposed drug product are the same as those of the reference listed drug.

(B) A reference to the applicant's annotated proposed labeling and to the currently approved labeling for the reference listed drug provided under paragraph (a)(8) of this section.

(ii) If the route of administration, dosage form, or strength of the drug product differs from the reference listed drug and the abbreviated application is submitted under an approved

petition under § 314.93, such information about the different route of administration, dosage form, or strength that FDA may require.

(7) *Bioequivalence.* (i) Information that shows that the drug product is bioequivalent to the reference listed drug upon which the applicant relies; or

(ii) If the abbreviated new drug application is submitted under a petition approved under § 314.93, the results of any bioavailability of bioequivalence testing required by the agency, or any other information required by the agency to show that the active ingredients of the proposed drug product are of the same pharmacological or therapeutic class as those in the reference listed drug and that the proposed drug product can be expected to have the same therapeutic effect as the reference listed drug. If the proposed drug product contains a different active ingredient than the reference listed drug, FDA will consider the proposed drug product to have the same therapeutic effect as the reference listed drug if the applicant provides information demonstrating that:

(A) There is an adequate scientific basis for determining that substitution of the specific proposed dose of the different active ingredient for the dose of the member of the same pharmacological or therapeutic class in the reference listed drug will yield a resulting drug product whose safety and effectiveness have not been adversely affected.

(B) The unchanged active ingredients in the proposed drug product are bioequivalent to those in the reference listed drug.

(C) The different active ingredient in the proposed drug product is bioequivalent to an approved dosage form containing that ingredient and approved for the same indication as the proposed drug product or is bioequivalent to a drug product offered for that indication which does not meet the definition of ''new drug'' under section 201(p) of the act.

(iii) For each in vivo bioequivalence study contained in the abbreviated new drug application, a description of the analytical and statistical methods used in each study and a statement with respect to each study that it either was conducted in compliance with the institutional review board regulations in part 56 of this chapter, or was not subject to the regulations under § 56.104 or § 56.105 of this chapter and that each study was conducted in compliance with the informed consent regulations in part 50 of this chapter.

(8) *Labeling*—(i) *Listed drug labeling.* A copy of the currently approved labeling for the listed drug referred to in the abbreviated new drug application, if the abbreviated new drug application relies on a reference listed drug.

(ii) *Proposed labeling.* Copies of the label and all labeling for the drug product (4 copies of draft labeling or 12 copies of final printed labeling).

(iii) A statement that the applicant's proposed labeling is the same as the labeling of the reference listed drug except for differences annotated and explained under paragraph (a)(8)(iv) of this section.

(iv) A side-by-side comparison of the applicant's proposed labeling with the approved labeling for the reference listed drug with all differences annotated and explained. Labeling (including the container label and package insert) proposed for the drug product must be the same as the labeling approved for the reference listed drug, except for changes required because of differences approved under a petition filed under § 314.93 or because the drug product and the reference listed drug are produced or distributed by different manufacturers. Such differences between the applicant's proposed labeling and labeling approved for the reference listed drug may include differences in expiration date, formulation, bioavailability, or pharmacokinetics, labeling revisions made to comply with current FDA labeling guidelines or other guidance, or omission of an indication or other aspect of labeling protected by patent or accorded exclusivity under section 505(j)(4)(D) of the act.

(9) *Chemistry, manufacturing, and controls.* (i) The information required under § 314.50(d)(1), except that § 314.50(d)(1)(ii)(*c*) shall contain the proposed or actual master production record, including a description of the

equipment, to be used for the manufacture of a commercial lot of the drug product.

(ii) *Inactive ingredients.* Unless otherwise stated in paragraphs (a)(9)(iii) through (a)(9)(v) of this section, an applicant shall identify and characterize the inactive ingredients in the proposed drug product and provide information demonstrating that such inactive ingredients do not affect the safety of the proposed drug product.

(iii) *Inactive ingredient changes permitted in drug products intended for parenteral use.* Generally, a drug product intended for parenteral use shall contain the same inactive ingredients and in the same concentration as the reference listed drug identified by the applicant under paragraph (a)(3) of this section. However, an applicant may seek approval of a drug product that differs from the reference listed drug in preservative, buffer, or antioxidant provided that the applicant identifies and characterizes the differences and provides information demonstrating that the differences do not affect the safety of the proposed drug product.

(iv) *Inactive ingredient changes permitted in drug products intended for ophthalmic or otic use.* Generally, a drug product intended for ophthalmic or otic use shall contain the same inactive ingredients and in the same concentration as the reference listed drug identified by the applicant under paragraph (a)(3) of this section. However, an applicant may seek approval of a drug product that differs from the reference listed drug in preservative, buffer, substance to adjust tonicity, or thickening agent provided that the applicant identifies and characterizes the differences and provides information demonstrating that the differences do not affect the safety of the proposed drug product, except that, in a product intended for ophthalmic use, an applicant may not change a buffer or substance to adjust tonicity for the purpose of claiming a therapeutic advantage over or difference from the listed drug, e.g., by using a balanced salt solution as a diluent as opposed to an isotonic saline solution, or by making a significant change in the pH or other change that may raise questions of irritability.

(v) *Inactive ingredient changes permitted in drug products intended for topical use.* Generally, a drug product intended for topical use shall contain the same inactive ingredients as the reference listed drug identified by the applicant under paragraph (a)(3) of this section. However, an applicant may seek approval of a drug product that differs from the reference listed drug provided that the applicant identifies and characterizes the differences and provides information demonstrating that the differences do not affect the safety of the proposed drug product.

(10) *Samples.* The information required under § 314.50(e)(1) and (e)(2)(i). Samples need not be submitted until requested by FDA.

(11) *Other.* The information required under § 314.50(g).

(12) *Patent certification*—(i) *Patents claiming drug, drug product, or method of use.* (A) Except as provided in paragraph (a)(12)(iv) of this section, a certification with respect to each patent issued by the United States Patent and Trademark Office that, in the opinion of the applicant and to the best of its knowledge, claims the reference listed drug or that claims a use of such listed drug for which the applicant is seeking approval under section 505(j) of the act and for which information is required to be filed under section 505(b) and (c) of the act and § 314.53. For each such patent, the applicant shall provide the patent number and certify, in its opinion and to the best of its knowledge, one of the following circumstances:

(*1*) That the patent information has not been submitted to FDA. The applicant shall entitle such a certification ''Paragraph I Certification'';

(*2*) That the patent has expired. The applicant shall entitle such a certification ''Paragraph II Certification'';

(*3*) The date on which the patent will expire. The applicant shall entitle such a certification ''Paragraph III Certification''; or

(*4*) That the patent is invalid, unenforceable, or will not be infringed by the manufacture, use, or sale of the drug product for which the abbreviated application is submitted. The applicant shall entitle such a certification

''Paragraph IV Certification''. This certification shall be submitted in the following form:

I, (*name of applicant*), certify that Patent No. _____ (*is invalid, unenforceable, or will not be infringed by the manufacture, use, or sale of*) (*name of proposed drug product*) for which this application is submitted.

The certification shall be accompanied by a statement that the applicant will comply with the requirements under § 314.95(a) with respect to providing a notice to each owner of the patent or their representatives and to the holder of the approved application for the listed drug, and with the requirements under § 314.95(c) with respect to the content of the notice.

(B) If the abbreviated new drug application refers to a listed drug that is itself a licensed generic product of a patented drug first approved under section 505(b) of the act, the appropriate patent certification under paragraph (a)(12)(i) of this section with respect to each patent that claims the first-approved patented drug or that claims a use for such drug.

(ii) *No relevant patents.* If, in the opinion of the applicant and to the best of its knowledge, there are no patents described in paragraph (a)(12)(i) of this section, a certification in the following form:

In the opinion and to the best knowledge of (*name of applicant*), there are no patents that claim the listed drug referred to in this application or that claim a use of the listed drug.

(iii) *Method of use patent.* (A) If patent information is submitted under section 505(b) or (c) of the act and § 314.53 for a patent claiming a method of using the listed drug, and the labeling for the drug product for which the applicant is seeking approval does not include any indications that are covered by the use patent, a statement explaining that the method of use patent does not claim any of the proposed indications.

(B) If the labeling of the drug product for which the applicant is seeking approval includes an indication that, according to the patent information submitted under section 505(b) or (c) of the act and § 314.53 or in the opinion of the applicant, is claimed by a use patent, an applicable certification under paragraph (a)(12)(i) of this section.

(iv) *Method of manufacturing patent.* An applicant is not required to make a certification with respect to any patent that claims only a method of manufacturing the listed drug.

(v) *Licensing agreements.* If the abbreviated new drug application is for a drug or method of using a drug claimed by a patent and the applicant has a licensing agreement with the patent owner, a certification under paragraph (a)(12)(i)(A)(*4*) of this section (''Paragraph IV Certification'') as to that patent and a statement that it has been granted a patent license.

(vi) *Late submission of patent information.* If a patent on the listed drug is issued and the holder of the approved application for the listed drug does not submit the required information on the patent within 30 days of issuance of the patent, an applicant who submitted an abbreviated new drug application for that drug that contained an appropriate patent certification before the submission of the patent information is not required to submit an amended certification. An applicant whose abbreviated new drug application is submitted after a late submission of patent information, or whose pending abbreviated application was previously submitted but did not contain an appropriate patent certification at the time of the patent submission, shall submit a certification under paragraph (a)(12)(i) of this section or a statement under paragraph (a)(12)(iii) of this section as to that patent.

(vii) *Disputed patent information.* If an applicant disputes the accuracy or relevance of patent information submitted to FDA, the applicant may seek a confirmation of the correctness of the patent information in accordance with the procedures under § 314.53(f). Unless the patent information is withdrawn or changed, the applicant shall submit an appropriate certification for each relevant patent.

(viii) *Amended certifications.* A certification submitted under paragraphs (a)(12)(i) through (a)(12)(iii) of this section may be amended at any time before the effective date of the approval of the application. However, an applicant who has submitted a paragraph IV

patent certification may not change it to a paragraph III certification if a patent infringement suit has been filed against another paragraph IV applicant unless the agency has determined that no applicant is entitled to 180-day exclusivity or the patent expires before the lawsuit is resolved or expires after the suit is resolved but before the end of the 180-day exclusivity period. If an applicant with a pending application voluntarily makes a patent certification for an untimely filed patent, the applicant may withdraw the patent certification for the untimely filed patent. An applicant shall submit an amended certification by letter or as an amendment to a pending application or by letter to an approved application. Once an amendment or letter is submitted, the application will no longer be considered to contain the prior certification.

(A) *After finding of infringement.* An applicant who has submitted a certification under paragraph (a)(12)(i)(A)(*4*) of this section and is sued for patent infringement within 45 days of the receipt of notice sent under § 314.95 shall amend the certification if a final judgment in the action against the applicant is entered finding the patent to be infringed. In the amended certification, the applicant shall certify under paragraph (a)(12)(i)(A)(*3*) of this section that the patent will expire on a specific date. Once an amendment or letter for the change has been submitted, the application will no longer be considered to be one containing a certification under paragraph (a)(12)(i)(A)(*4*) of this section. If a final judgment finds the patent to be invalid and infringed, an amended certification is not required.

(B) *After removal of a patent from the list.* If a patent is removed from the list, any applicant with a pending application (including a tentatively approved application with a delayed effective date) who has made a certification with respect to such patent shall amend its certification. The applicant shall certify under paragraph (a)(12)(ii) of this section that no patents described in paragraph (a)(12)(i) of this section claim the drug or, if other relevant patents claim the drug, shall amend the certification to refer only to those relevant patents. In the amendment, the applicant shall state the reason for the change in certification (that the patent is or has been removed from the list). A patent that is the subject of a lawsuit under § 314.107(c) shall not be removed from the list until FDA determines either that no delay in effective dates of approval is required under that section as a result of the lawsuit, that the patent has expired, or that any such period of delay in effective dates of approval is ended. An applicant shall submit an amended certification. Once an amendment or letter for the change has been submitted, the application will no longer be considered to be one containing a certification under paragraph (a)(12)(i)(A)(4) of this section.

(C) *Other amendments.* (*1*) Except as provided in paragraphs (a)(12)(vi) and (a)(12)(viii)(C)(*2*) of this section, an applicant shall amend a submitted certification if, at any time before the effective date of the approval of the application, the applicant learns that the submitted certification is no longer accurate.

(*2*) An applicant is not required to amend a submitted certification when information on a patent on the listed drug is submitted after the effective date of approval of the abbreviated application.

(13) *Financial certification or disclosure statement.* An abbreviated application shall contain a financial certification or disclosure statement as required by part 54 of this chapter.

(b) *Drug products subject to the Drug Efficacy Study Implementation (DESI) review.* If the abbreviated new drug application is for a duplicate of a drug product that is subject to FDA's DESI review (a review of drug products approved as safe between 1938 and 1962) or other DESI-like review and the drug product evaluated in the review is a listed drug, the applicant shall comply with the provisions of paragraph (a) of this section.

(c) *Abbreviated antibiotic application.* For applications submitted under section 507 of the act, the applicant shall submit a complete archival copy of the abbreviated application that contains the information described under § 314.50 (a)(1), (a)(3), (a)(4), and (a)(5), (b), (d)(1) and (d)(3), (e), and (g). The applicant

shall state whether the submission is an abbreviated application under this section or a supplement to an abbreviated application under § 314.97.

(d) *Format of an abbreviated application.* (1) The applicant shall submit a complete archival copy of the abbreviated application as required under paragraphs (a) and (c) of this section. FDA will maintain the archival copy during the review of the application to permit individual reviewers to refer to information that is not contained in their particular technical sections of the application, to give other agency personnel access to the application for official business, and to maintain in one place a complete copy of the application. An applicant may submit all or portions of the archival copy of the abbreviated application in any form (e.g., microfiche, optical disc, and magnetic tape) that the applicant and FDA agree is acceptable.

(2) For abbreviated new drug applications, the applicant shall submit a review copy of the abbreviated application that contains two separate sections. One section shall contain the information described under paragraphs (a)(2) through (a)(6), (a)(8), and (a)(9) of this section 505(j)(2)(A)(vii) of the act and one copy of the analytical methods and descriptive information needed by FDA's laboratories to perform tests on samples of the proposed drug product and to validate the applicant's analytical methods. The other section shall contain the information described under paragraphs (a)(3), (a)(7), and (a)(8) of this section. Each of the sections in the review copy is required to contain a copy of the application form described under § 314.50(a).

(3) For abbreviated antibiotic applications, the applicant shall submit a review copy that contains the technical sections described in § 314.50 (d)(1) and (d)(3). Each of the technical sections in the review copy is required to be separate with a copy of the application form required under § 314.50(a).

(4) The applicant may obtain from FDA sufficient folders to bind the archival, the review, and the field copies of the abbreviated application.

(5) The applicant shall submit a field copy of the abbreviated application that contains the technical section described in paragraph (a)(9) of this section, a copy of the application form required under paragraph (a)(1) of this section, and a certification that the field copy is a true copy of the technical section described in paragraph (a)(9) of this section contained in the archival and review copies of the abbreviated application.

[57 FR 17983, Apr. 28, 1992; 57 FR 29353, July 1, 1992, as amended at 58 FR 47352, Sept. 8, 1993; 59 FR 50364, Oct. 3, 1994; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 314.94 was amended by adding new paragraph (a)(13), effective Feb. 2, 1999.

§ 314.95 Notice of certification of invalidity or noninfringement of a patent.

(a) *Notice of certification.* For each patent that claims the listed drug or that claims a use for such listed drug for which the applicant is seeking approval and that the applicant certifies under § 314.94(a)(12) is invalid, unenforceable, or will not be infringed, the applicant shall send notice of such certification by registered or certified mail, return receipt requested to each of the following persons:

(1) Each owner of the patent which is the subject of the certification or the representative designated by the owner to receive the notice. The name and address of the patent owner or its representative may be obtained from the United States Patent and Trademark Office; and

(2) The holder of the approved application under section 505(b) of the act for the listed drug that is claimed by the patent and for which the applicant is seeking approval, or, if the application holder does not reside or maintain a place of business within the United States, the application holder's attorney, agent, or other authorized official. The name and address of the application holder or its attorney, agent, or authorized official may be obtained from the Division of Drug Information Resources (HFD–80), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(3) This paragraph does not apply to a use patent that claims no uses for

which the applicant is seeking approval.

(b) *Sending the notice.* The applicant shall send the notice required by paragraph (a) of this section when it receives from FDA an acknowledgment letter stating that its abbreviated new drug application is sufficiently complete to permit a substantive review. At the same time, the applicant shall amend its abbreviated new drug application to include a statement certifying that the notice has been provided to each person identified under paragraph (a) of this section and that the notice met the content requirements under paragraph (c) of this section.

(c) *Contents of a notice.* In the notice, the applicant shall cite section 505(j)(2)(B)(ii) of the act and shall include, but not be limited to, the following information:

(1) A statement that FDA has received an abbreviated new drug application submitted by the applicant containing any required bioavailability or bioequivalence data or information.

(2) The abbreviated application number.

(3) The established name, if any, as defined in section 502(e)(3) of the act, of the proposed drug product.

(4) The active ingredient, strength, and dosage form of the proposed drug product.

(5) The patent number and expiration date, as submitted to the agency or as known to the applicant, of each patent alleged to be invalid, unenforceable, or not infringed.

(6) A detailed statement of the factual and legal basis of the applicant's opinion that the patent is not valid, unenforceable, or will not be infringed. The applicant shall include in the detailed statement:

(i) For each claim of a patent alleged not to be infringed, a full and detailed explanation of why the claim is not infringed.

(ii) For each claim of a patent alleged to be invalid or unenforceable, a full and detailed explanation of the grounds supporting the allegation.

(7) If the applicant does not reside or have a place of business in the United States, the name and address of an agent in the United States authorized to accept service of process for the applicant.

(d) *Amendment to an abbreviated application.* If an abbreviated application is amended to include the certification described in § 314.94(a)(12)(i)(A)(*4*), the applicant shall send the notice required by paragraph (a) of this section at the same time that the amendment to the abbreviated application is submitted to FDA.

(e) *Documentation of receipt of notice.* The applicant shall amend its abbreviated application to document receipt of the notice required under paragraph (a) of this section by each person provided the notice. The applicant shall include a copy of the return receipt or other similar evidence of the date the notification was received. FDA will accept as adequate documentation of the date of receipt a return receipt or a letter acknowledging receipt by the person provided the notice. An applicant may rely on another form of documentation only if FDA has agreed to such documentation in advance. A copy of the notice itself need not be submitted to the agency.

(f) *Approval.* If the requirements of this section are met, FDA will presume the notice to be complete and sufficient, and it will count the day following the date of receipt of the notice by the patent owner or its representative and by the approved application holder as the first day of the 45-day period provided for in section 505(j)(4)(B)(iii) of the act. FDA may, if the applicant provides a written statement to FDA that a later date should be used, count from such later date.

[59 FR 50366, Oct. 3, 1994]

§ 314.96 Amendments to an unapproved abbreviated application.

(a) *Abbreviated new drug application.* (1) An applicant may amend an abbreviated new drug application that is submitted under § 314.94, but not yet approved, to revise existing information or provide additional information.

(2) Submission of an amendment containing significant data or information constitutes an agreement between FDA and the applicant to extend the review period only for the time necessary to review the significant data or information and for no more than 180 days.

(3) Submission of an amendment containing significant data or information to resolve deficiencies in the application as set forth in a not approvable letter issued under § 314.120 constitutes an agreement between FDA and the applicant under section 505(j)(4)(A) of the act to extend the date by which the agency is required to reach a decision on the abbreviated new drug application only for the time necessary to review the significant data or information and for no more than 180 days.

(b) The applicant shall submit a field copy of each amendment to § 314.94(a)(9). The applicant, other than a foreign applicant, shall include in its submission of each such amendment to FDA a statement certifying that a field copy of the amendment has been sent to the applicant's home FDA district office.

(c) *Abbreviated antibiotic application.* The applicant shall comply with the provisions of § 314.60.

[57 FR 17983, Apr. 28, 1992, as amended at 58 FR 47352, Sept. 8, 1993]

§ 314.97 Supplements and other changes to an approved abbreviated application.

The applicant shall comply with the requirements of §§ 314.70 and 314.71 regarding the submission of supplemental applications and other changes to an approved abbreviated application.

§ 314.98 Postmarketing reports.

(a) Except as provided in paragraph (b) of this section, each applicant having an approved abbreviated antibiotic application under § 314.94 or approved abbreviated new drug application under § 314.94 that is effective shall comply with the requirements of § 314.80 regarding the reporting and recordkeeping of adverse drug experiences.

(b) Each applicant shall submit one copy of each report required under § 314.80 to the Division of Epidemiology and Surveillance (HFD–730), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

(c) Each applicant shall make the reports required under § 314.81 and sections 505(k) and 507(g) of the act for each of its approved abbreviated applications.

§ 314.99 Other responsibilities of an applicant of an abbreviated application.

(a) An applicant shall comply with the requirements of § 314.65 regarding withdrawal by the applicant of an unapproved abbreviated application and § 314.72 regarding a change in ownership of an abbreviated application.

(b) An applicant may ask FDA to waive under this section any requirement that applies to the applicant under §§ 314.92 through 314.99. The applicant shall comply with the requirements for a waiver under § 314.90.

Subpart D—FDA Action on Applications and Abbreviated Applications

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted. Redesignated at 57 FR 17983, Apr. 28, 1992.

§ 314.100 Timeframes for reviewing applications and abbreviated applications.

(a) Within 180 days of receipt of an application for a new drug under section 505(b) of the act, or of an abbreviated application for a new drug under section 505(j) of the act, or of an application or abbreviated application for an antibiotic drug under section 507 of the act, FDA will review it and send the applicant either an approval letter under § 314.105, or an approvable letter under § 314.110, or a not approvable letter under § 314.120. This 180-day period is called the ''review clock.''

(b) During the review period, an applicant may withdraw an application under § 314.65 or an abbreviated application under § 314.99 and later resubmit it. FDA will treat the resubmission as a new application or abbreviated application.

(c) The review clock may be extended by mutual agreement between FDA and an applicant or as provided in §§ 314.60 and 314.96, as the result of a major amendment.

[57 FR 17987, Apr. 28, 1992]

§ 314.101 Filing an application and an abbreviated antibiotic application and receiving an abbreviated new drug application.

(a)(1) Within 60 days after FDA receives an application or abbreviated antibiotic application, the agency will determine whether the application or abbreviated antibiotic application may be filed. The filing of an application or abbreviated antibiotic application means that FDA has made a threshold determination that the application or abbreviated antibiotic application is sufficiently complete to permit a substantive review.

(2) If FDA finds that none of the reasons in paragraphs (d) and (e) of this section for refusing to file the application or abbreviated antibiotic apply, the agency will file the application or abbreviated antibiotic application and notify the applicant in writing. The date of filing will be the date 60 days after the date FDA received the application or abbreviated antibiotic application. The date of filing begins the 180-day period described in section 505(c) of the act. This 180-day period is called the ''filing clock.''

(3) If FDA refuses to file the application or abbreviated antibiotic application, the agency will notify the applicant in writing and state the reason under paragraph (d) or (e) of this section for the refusal. If FDA refuses to file the application or abbreviated antibiotic application under paragraph (d) of this section, the applicant may request in writing within 30 days of the date of the agency's notification an informal conference with the agency about whether the agency should file the application or abbreviated antibiotic application. If, following the informal conference, the applicant requests that FDA file the application or abbreviated antibiotic application (with or without amendments to correct the deficiencies), the agency will file the application or abbreviated antibiotic application over protest under paragraph (a)(2) of this section, notify the applicant in writing, and review it as filed. If the application or abbreviated antibiotic application is filed over protest, the date of filing will be the date 60 days after the date the applicant requested the informal conference. The applicant need not resubmit a copy of an application or abbreviated antibiotic application that is filed over protest. If FDA refuses to file the application or abbreviated antibiotic application under paragraph (e) of this section, the applicant may amend the application or abbreviated antibiotic application and resubmit it, and the agency will make a determination under this section whether it may be filed.

(b)(1) An abbreviated new drug application will be reviewed after it is submitted to determine whether the abbreviated application may be received. Receipt of an abbreviated new drug application means that FDA has made a threshold determination that the abbreviated application is sufficiently complete to permit a substantive review.

(2) If FDA finds that none of the reasons in paragraphs (d) and (e) of this section for considering the abbreviated new drug application not to have been received applies, the agency will receive the abbreviated new drug application and notify the applicant in writing.

(3) If FDA considers the abbreviated new drug application not to have been received under paragraph (d) or (e) of this section, FDA will notify the applicant, ordinarily by telephone. The applicant may then:

(i) Withdraw the abbreviated new drug application under § 314.99; or

(ii) Amend the abbreviated new drug application to correct the deficiencies; or

(iii) Take no action, in which case FDA will refuse to receive the abbreviated new drug application.

(c) [Reserved]

(d) FDA may refuse to file an application or abbreviated antibiotic application or may not consider an abbreviated new drug application to be received if any of the following applies:

(1) The application or abbreviated application does not contain a completed application form.

(2) The application or abbreviated application is not submitted in the form required under § 314.50 or § 314.94.

(3) The application or abbreviated application is incomplete because it does

not on its face contain information required under section 505(b), section 505(j), or section 507 of the act and § 314.50 or § 314.94.

(4) The applicant fails to submit a complete environmental assessment, which addresses each of the items specified in the applicable format under § 25.40 of this chapter or fails to provide sufficient information to establish that the requested action is subject to categorical exclusion under § 25.30 or § 25.31 of this chapter.

(5) The application or abbreviated application does not contain an accurate and complete English translation of each part of the application that is not in English.

(6) The application does not contain a statement for each nonclinical laboratory study that it was conducted in compliance with the requirements set forth in part 58 of this chapter, or, for each study not conducted in compliance with part 58 of this chapter, a brief statement of the reason for the noncompliance.

(7) The application does not contain a statement for each clinical study that it was conducted in compliance with the institutional review board regulations in part 56 of this chapter, or was not subject to those regulations, and that it was conducted in compliance with the informed consent regulations in part 50 of this chapter, or, if the study was subject to but was not conducted in compliance with those regulations, the application does not contain a brief statement of the reason for the noncompliance.

(8) The drug product that is the subject of the submission is already covered by an approved application or abbreviated application and the applicant of the submission:

(i) Has an approved application or abbreviated application for the same drug product; or

(ii) Is merely a distributor and/or repackager of the already approved drug product.

(9) The application is submitted as a 505(b)(2) application for a drug that is a duplicate of a listed drug and is eligible for approval under section 505(j) of the act.

(e) The agency will refuse to file an application or abbreviated antibiotic application or will consider an abbreviated new drug application not to have been received if any of the following applies:

(1) The drug product is subject to licensing by FDA under the Public Health Service Act (42 U.S.C. 201 *et seq.*) and subchapter F of this chapter.

(2) In the case of a 505(b)(2) application or an abbreviated new drug application, the drug product contains the same active moiety as a drug that:

(i) Was approved after September 24, 1984, in an application under section 505(b) of the act, and

(ii) Is entitled to a 5-year period of exclusivity under section 505(c)(3)(D)(ii) and (j)(4)(D)(ii) of the act and § 314.108(b)(2), unless the 5-year exclusivity period has elapsed or unless 4 years of the 5-year period have elapsed and the application or abbreviated application contains a certification of patent invalidity or noninfringement described in § 314.50(i)(1)(i)(A)(*4*) or § 314.94(a)(12)(i)(A)(*4*).

(f)(1) Within 180 days after the date of filing, plus the period of time the review period was extended (if any), FDA will either:

(i) Approve the application or abbreviated antibiotic application; or

(ii) Issue a notice of opportunity for hearing if the applicant asked FDA to provide it an opportunity for a hearing on an application or abbreviated antibiotic application in response to an approvable letter or a not approvable letter.

(2) Within 180 days after the date of receipt, plus the period of time the review clock was extended (if any), FDA will either approve or disapprove the abbreviated new drug application. If FDA disapproves the abbreviated new drug application, FDA will issue a notice of opportunity for hearing if the applicant asked FDA to provide it an opportunity for a hearing on an abbreviated new drug application in response to a not approvable letter.

(3) This paragraph does not apply to applications or abbreviated applications that have been withdrawn from FDA review by the applicant.

[57 FR 17987, Apr. 28, 1992; 57 FR 29353, July 1, 1992, as amended at 59 FR 50366, Oct. 3, 1994; 62 FR 40599, July 29, 1997]

§ 314.102 Communications between FDA and applicants.

(a) *General principles.* During the course of reviewing an application or an abbreviated application, FDA shall communicate with applicants about scientific, medical, and procedural issues that arise during the review process. Such communication may take the form of telephone conversations, letters, or meetings, whichever is most appropriate to discuss the particular issue at hand. Communications shall be appropriately documented in the application in accordance with § 10.65 of this chapter. Further details on the procedures for communication between FDA and applicants are contained in a staff manual guide that is publicly available.

(b) *Notification of easily correctable deficiencies.* FDA reviewers shall make every reasonable effort to communicate promptly to applicants easily correctable deficiencies found in an application or an abbreviated application when those deficiencies are discovered, particularly deficiencies concerning chemistry, manufacturing, and controls issues. The agency will also inform applicants promptly of its need for more data or information or for technical changes in the application or the abbreviated application needed to facilitate the agency's review. This early communication is intended to permit applicants to correct such readily identified deficiencies relatively early in the review process and to submit an amendment before the review period has elapsed. Such early communication would not ordinarily apply to major scientific issues, which require consideration of the entire pending application or abbreviated application by agency managers as well as reviewing staff. Instead, major scientific issues will ordinarily be addressed in an action letter.

(c) *Ninety-day conference.* Approximately 90 days after the agency receives the application, FDA will provide applicants with an opportunity to meet with agency reviewing officials. The purpose of the meeting will be to inform applicants of the general progress and status of their applications, and to advise applicants of deficiencies that have been identified by that time and that have not already been communicated. This meeting will be available on applications for all new chemical entities and major new indications of marketed drugs. Such meetings will be held at the applicant's option, and may be held by telephone if mutually agreed upon. Such meetings would not ordinarily be held on abbreviated applications because they are not submitted for new chemical entities or new indications.

(d) *End of review conference.* At the conclusion of FDA's review of an application or an abbreviated application as designated by the issuance of an approvable or not approvable letter, FDA will provide applicants with an opportunity to meet with agency reviewing officials. The purpose of the meeting will be to discuss what further steps need to be taken by the applicant before the application or abbreviated application can be approved. This meeting will be available on all applications or abbreviated applications, with priority given to applications for new chemical entities and major new indications for marketed drugs and for the first duplicates for such drugs. Requests for such meetings shall be directed to the director of the division responsible for reviewing the application or abbreviated application.

(e) *Other meetings.* Other meetings between FDA and applicants may be held, with advance notice, to discuss scientific, medical, and other issues that arise during the review process. Requests for meetings shall be directed to the director of the division responsible for reviewing the application or abbreviated application. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times. However, ''drop-in'' visits (i.e., an unannounced and unscheduled visit by a company representative) are discouraged except for urgent matters, such as to discuss an important new safety issue.

[57 FR 17988, Apr. 28, 1992; 57 FR 29353, July 1, 1992]

§ 314.103 Dispute resolution.

(a) *General.* FDA is committed to resolving differences between applicants

and FDA reviewing divisions with respect to technical requirements for applications or abbreviated applications as quickly and amicably as possible through the cooperative exchange of information and views.

(b) *Administrative and procedural issues.* When administrative or procedural disputes arise, the applicant should first attempt to resolve the matter with the division responsible for reviewing the application or abbreviated application, beginning with the consumer safety officer assigned to the application or abbreviated application. If resolution is not achieved, the applicant may raise the matter with the person designated as ombudsman, whose function shall be to investigate what has happened and to facilitate a timely and equitable resolution. Appropriate issues to raise with the ombudsman include resolving difficulties in scheduling meetings, obtaining timely replies to inquiries, and obtaining timely completion of pending reviews. Further details on this procedure are contained in a staff manual guide that is publicly available under FDA's public information regulations in part 20.

(c) *Scientific and medical disputes.* (1) Because major scientific issues are ordinarily communicated to applicants in an approvable or not approvable letter pursuant to § 314.110 or § 314.120, respectively, the ''end-of-review conference'' described in § 314.102(d) will provide a timely forum for discussing and resolving, if possible, scientific and medical issues on which the applicant disagrees with the agency. In addition, the ''ninety-day conference'' described in § 314.102(c) will provide a timely forum for discussing and resolving, if possible, issues identified by that date.

(2) When scientific or medical disputes arise at other times during the review process, applicants should discuss the matter directly with the responsible reviewing officials. If necessary, applicants may request a meeting with the appropriate reviewing officials and management representatives in order to seek a resolution. Ordinarily, such meetings would be held first with the Division Director, then with the Office Director, and finally with the Center Director if the matter is still unresolved. Requests for such meetings shall be directed to the director of the division responsible for reviewing the application or abrreviated application. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times.

(3) In requesting a meeting designed to resolve a scientific or medical dispute, applicants may suggest that FDA seek the advice of outside experts, in which case FDA may, in its discretion, invite to the meeting one or more of its advisory committee members or other consultants, as designated by the agency. Applicants may also bring their own consultants. For major scientific and medical policy issues not resolved by informal meetings, FDA may refer the matter to one of its standing advisory committees for its consideration and recommendations.

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 57 FR 17989, Apr. 28, 1992]

§ 314.104 Drugs with potential for abuse.

The Food and Drug Administration will inform the Drug Enforcement Administration under section 201(f) of the Controlled Substances Act (21 U.S.C. 801) when an application or abbreviated application is submitted for a drug that appears to have an abuse potential.

[57 FR 17989, Apr. 28, 1992]

§ 314.105 Approval of an application and an abbreviated application.

(a) The Food and Drug Administration will approve an application or an abbreviated antibiotic application and sent the applicant an approval letter if none of the reasons in § 314.125 for refusing to approve the application or abbreviated antibiotic application applies. An approval becomes effective on the date of the issuance of the approval letter, except with regard to an approval under section 505(b)(2) of the act with a delayed effective date. An approval with a delayed effective date is tentative and does not become final until the effective date. When FDA sends an applicant an approval letter for an antibiotic, it will promulgate a regulation under § 314.300 providing for

certification of the drug, if necessary. A new drug product or antibiotic approved under this paragraph may not be marketed until an approval is effective. Marketing of an antibiotic need not await the promulgation of a regulation under § 314.300.

(b) FDA will approve an application or abbreviated antibiotic application and issue the applicant an approval letter (rather than an approvable letter under § 314.110) on the basis of draft labeling if the only deficiencies in the application or abbreviated antibiotic application concern editorial or similar minor deficiencies in the draft labeling. Such approval will be conditioned upon the applicant incorporating the specified labeling changes exactly as directed, and upon the applicant submitting to FDA a copy of the final printed labeling prior to marketing.

(c) FDA will approve an application after it determines that the drug meets the statutory standards for safety and effectiveness, manufacturing and controls, and labeling, and an abbreviated application after it determines that the drug meets the statutory standards for manufacturing and controls, labeling, and, where applicable, bioequivalence. While the statutory standards apply to all drugs, the many kinds of drugs that are subject to the statutory standards and the wide range of uses for those drugs demand flexibility in applying the standards. Thus FDA is required to exercise its scientific judgment to determine the kind and quantity of data and information an applicant is required to provide for a particular drug to meet the statutory standards. FDA makes its views on drug products and classes of drugs available through guidelines, recommendations, and other statements of policy.

(d) FDA will approve an abbreviated new drug application and send the applicant an approval letter if none of the reasons in § 314.127 for refusing to approve the abbreviated new drug application applies. The approval becomes effective on the date of the issuance of the agency's approval letter unless the approval letter provides for a delayed effective date. An approval with a delayed effective date is tentative and does not become final until the effective date. A new drug product approved under this paragraph may not be introduced or delivered for introduction into interstate commerce until approval of the abbreviated new drug application is effective. Ordinarily, the effective date of approval will be stated in the approval letter.

[57 FR 17989, Apr. 28, 1992]

§ 314.106 Foreign data.

(a) *General.* The acceptance of foreign data in an application generally is governed by § 312.120 of this chapter.

(b) *As sole basis for marketing approval.* An application based solely on foreign clinical data meeting U.S. criteria for marketing approval may be approved if: (1) The foreign data are applicable to the U.S. population and U.S. medical practice; (2) the studies have been performed by clinical investigators of recognized competence; and (3) the data may be considered valid without the need for an on-site inspection by FDA or, if FDA considers such an inspection to be necessary, FDA is able to validate the data through an on-site inspection or other appropriate means. Failure of an application to meet any of these criteria will result in the application not being approvable based on the foreign data alone. FDA will apply this policy in a flexible manner according to the nature of the drug and the data being considered.

(c) *Consultation between FDA and applicants.* Applicants are encouraged to meet with agency officials in a ''presubmission'' meeting when approval based solely on foreign data will be sought.

[50 FR 7493, Feb. 22, 1985, as amended at 55 FR 11580, Mar. 29, 1990]

§ 314.107 Effective date of approval of a 505(b)(2) application or abbreviated new drug application under section 505(j) of the act.

(a) *General.* A drug product may be introduced or delivered for introduction into interstate commerce when approval of the application or abbreviated application for the drug product becomes effective. Except as provided in this section, approval of an application or abbreviated application for a drug product becomes effective on the date FDA issues an approval letter

under § 314.105 for the application or abbreviated application.

(b) *Effect of patent on the listed drug.* If approval of an abbreviated new drug application submitted under section 505(j) of the act or of a 505(b)(2) application is granted, that approval will become effective in accordance with the following:

(1) *Date of approval letter.* Except as provided in paragraphs (b)(3), (b)(4), and (c) of this section, approval will become effective on the date FDA issues an approval letter under § 314.105 if the applicant certifies under § 314.50(i) or § 314.94(a)(12) that:

(i) There are no relevant patents; or

(ii) The applicant is aware of a relevant patent but the patent information required under section 505 (b) or (c) of the act has not been submitted to FDA; or

(iii) The relevant patent has expired; or

(iv) The relevant patent is invalid, unenforceable, or will not be infringed.

(2) *Patent expiration.* If the applicant certifies under § 314.50(i) or § 314.94(a)(12) that the relevant patent will expire on a specified date, approval will become effective on the specified date.

(3) *Disposition of patent litigation.* (i)(A) Except as provided in paragraphs (b)(3)(ii), (b)(3)(iii), and (b)(3)(iv) of this section, if the applicant certifies under § 314.50(i) or § 314.94(a)(12) that the relevant patent is invalid, unenforceable, or will not be infringed, and the patent owner or its representative or the exclusive patent licensee brings suit for patent infringement within 45 days of receipt by the patent owner of the notice of certification from the applicant under § 314.52 or § 314.95, approval may be made effective 30 months after the date of the receipt of the notice of certification by the patent owner or by the exclusive licensee (or their representatives) unless the court has extended or reduced the period because of a failure of either the plaintiff or defendant to cooperate reasonably in expediting the action; or

(B) If the patented drug product qualifies for 5 years of exclusive marketing under § 314.108(b)(2) and the patent owner or its representative or the exclusive patent licensee brings suit for patent infringement during the 1-year period beginning 4 years after the date the patented drug was approved and within 45 days of receipt by the patent owner of the notice of certification, the approval may be made effective at the expiration of the 7½ years from the date of approval of the application for the patented drug product.

(ii) If before the expiration of the 30-month period, or 7½ years where applicable, the court issues a final order that the patent is invalid, unenforceable, or not infringed, approval may be made effective on the date the court enters judgment;

(iii) If before the expiration of the 30-month period, or 7½ years where applicable, the court issues a final order or judgment that the patent has been infringed, approval may be made effective on the date the court determines that the patent will expire or otherwise orders; or

(iv) If before the expiration of the 30-month period, or 7½ years where applicable, the court grants a preliminary injunction prohibiting the applicant from engaging in the commercial manufacture or sale of the drug product until the court decides the issues of patent validity and infringement, and if the court later decides that the patent is invalid, unenforceable, or not infringed, approval may be made effective on the date the court enters a final order or judgment that the patent is invalid, unenforceable, or not infringed.

(v) In order for an approval to be made effective under paragraph (b)(3) of this section, the applicant must receive an approval letter from the agency indicating that the application has received final approval. Tentative approval of an application does not constitute ''approval'' of an application and cannot, absent a final approval letter from the agency, result in an effective approval under paragraph (b)(3) of this section.

(4) *Multiple certifications.* If the applicant has submitted certifications under § 314.50(i) or § 314.94(a)(12) for more than one patent, the date of approval will be calculated for each certification, and the approval will become effective on the last applicable date.

(c) *Subsequent abbreviated new drug application submission.* (1) If an abbreviated new drug application contains a certification that a relevant patent is invalid, unenforceable, or will not be infringed and the application is for a generic copy of the same listed drug for which one or more substantially complete abbreviated new drug applications were previously submitted containing a certification that the same patent was invalid, unenforceable, or would not be infringed and the applicant submitting the first application has successfully defended against a suit for patent infringement brought within 45 days of the patent owner's receipt of notice submitted under § 314.95, approval of the subsequent abbreviated new drug application will be made effective no sooner than 180 days from whichever of the following dates is earlier:

(i) The date the applicant submitting the first application first commences commercial marketing of its drug product; or

(ii) The date of a decision of the court holding the relevant patent invalid, unenforceable, or not infringed.

(2) For purposes of paragraph (c)(1) of this section, the ''applicant submitting the first application'' is the applicant that submits an application that is both substantially complete and contains a certification that the patent was invalid, unenforceable, or not infringed prior to the submission of any other application for the same listed drug that is both substantially complete and contains the same certification. A ''substantially complete'' application must contain the results of any required bioequivalence studies, or, if applicable, a request for a waiver of such studies.

(3) For purposes of paragraph (c)(1) of this section, if FDA concludes that the applicant submitting the first application is not actively pursuing approval of its abbreviated application, FDA will make the approval of subsequent abbreviated applications immediately effective if they are otherwise eligible for an immediately effective approval.

(4) For purposes of paragraph (c)(1)(i) of this section, the applicant submitting the first application shall, if sued for patent infringement, notify FDA of the date that it commences commercial marketing of its drug product. Commercial marketing commences with the first date of introduction or delivery for introduction into interstate commerce outside the control of the manufacturer of a drug product, except for investigational use under part 312 of this chapter, but does not include transfer of the drug product for reasons other than sale within the control of the manufacturer or application holder. If an applicant does not promptly notify FDA of such date, the effective date of approval shall be deemed to be the date of the commencement of first commercial marketing.

(d) *Delay due to exclusivity.* The agency will also delay the effective date of the approval of an abbreviated new drug application under section 505(j) of the act or a 505(b)(2) application if delay is required by the exclusivity provisions in § 314.108. When the effective date of an application is delayed under both this section and § 314.108, the effective date will be the later of the 2 days specified under this section and § 314.108.

(e) *Court actions.* (1) References to actions of ''the court'' in paragraphs (b) and (c) of this section are to the court that enters final judgment from which no appeal can be or has been taken.

(2) For purposes of establishing the effective date of approval based on a court judgment, the following dates shall be deemed to be the date of the final decision of the court on the patent issues:

(i) If the district court enters a decision that the patent is invalid, unenforceable, or not infringed, and the decision is not appealed, the date on which the right to appeal lapses.

(ii) If the district court enters a decision that the patent is invalid, unenforceable, or not infringed, and the decision is appealed, the date of the first decision or order by a higher court holding or affirming the decision of the district court that the patent is invalid, unenforceable, or not infringed.

(iii) If the district court enters a decision that the patent is infringed, and the decision is appealed, the date on

which the district court enters a judgment that the patent is invalid, unenforceable, or not infringed pursuant to a mandate issued by a court of appeals.

(iv) The applicant shall submit a copy of the entry of the order or judgment to the Office of Generic Drugs (HFD–600), or to the appropriate division in the Office of Drug Evaluation I (HFD–100) or Office of Drug Evaluation II (HFD–500), whichever is applicable, within 10 working days of a final judgment.

(f) *Computation of 45-day time clock.* (1) The 45-day clock described in paragraph (b)(3) of this section begins on the day after the date of receipt of the applicant's notice of certification by the patent owner or its representative, and by the approved application holder. When the 45th day falls on Saturday, Sunday, or a Federal holiday, the 45th day will be the next day that is not a Saturday, Sunday, or a Federal holiday.

(2) The abbreviated new drug applicant or the 505(b)(2) applicant shall notify FDA immediately of the filing of any legal action filed within 45 days of receipt of the notice of certification. If the applicant submitting the abbreviated new drug application or the 505(b)(2) application or patent owner or its representative does not notify FDA in writing before the expiration of the 45-day time period or the completion of the agency's review of the application, whichever occurs later, that a legal action for patent infringement was filed within 45 days of receipt of the notice of certification, approval of the abbreviated new drug application or the 505(b)(2) application will be made effective immediately upon expiration of the 45 days or upon completion of the agency's review and approval of the application, whichever is later. The notification to FDA of the legal action shall include:

(i) The abbreviated new drug application or 505(b)(2) application number.

(ii) The name of the abbreviated new drug or 505(b)(2) application applicant.

(iii) The established name of the drug product or, if no established name exists, the name(s) of the active ingredient(s), the drug product's strength, and dosage form.

(iv) A certification that an action for patent infringement identified by number, has been filed in an appropriate court on a specified date.

The applicant of an abbreviated new drug application shall send the notification to FDA's Office of Generic Drugs (HFD–600). A 505(b)(2) applicant shall send the notification to the appropriate division in the Center for Drug Evaluation and Research reviewing the application. A patent owner or its representative may also notify FDA of the filing of any legal action for patent infringement. The notice should contain the information and be sent to the offices or divisions described in this paragraph.

(3) If the patent owner or approved application holder who is an exclusive patent licensee waives its opportunity to file a legal action for patent infringement within 45 days of a receipt of the notice of certification and the patent owner or approved application holder who is an exclusive patent licensee submits to FDA a valid waiver before the 45 days elapse, approval of the abbreviated new drug application or the 505(b)(2) application will be made effective upon completion of the agency's review and approval of the application. FDA will only accept a waiver in the following form:

(*Name of patent owner or exclusive patent licensee*) has received notice from (*name of applicant*) under (*section 505(b)(3) or 505(j)(2)(B) of the act*) and does not intend to file an action for patent infringement against (*name of applicant*) concerning the drug (*name of drug*) before (*date on which 45 days elapses.* (*Name of patent owner or exclusive patent licensee*) waives the opportunity provided by (*section 505(c)(3)(C) or 505(j)(B)(iii) of the act*) and does not object to FDA's approval of (*name of applicant*)'s (*505(b)(2) or abbreviated new drug application*) for (*name of drug*) with an immediate effective date on or after the date of this letter.

[59 FR 50367, Oct. 3, 1994]

§ 314.108 New drug product exclusivity.

(a) *Definitions.* The following definitions of terms apply to this section:

Active moiety means the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or

other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance.

Approved under section 505(b) means an application submitted under section 505(b) and approved on or after October 10, 1962, or an application that was ''deemed approved'' under section 107(c)(2) of Pub. L. 87–781.

Clinical investigation means any experiment other than a bioavailability study in which a drug is administered or dispensed to, or used on, human subjects.

Conducted or sponsored by the applicant with regard to an investigation means that before or during the investigation, the applicant was named in Form FDA–1571 filed with FDA as the sponsor of the investigational new drug application under which the investigation was conducted, or the applicant or the applicant's predecessor in interest, provided substantial support for the investigation. To demonstrate ''substantial support,'' an applicant must either provide a certified statement from a certified public accountant that the applicant provided 50 percent or more of the cost of conducting the study or provide an explanation why FDA should consider the applicant to have conducted or sponsored the study if the applicant's financial contribution to the study is less than 50 percent or the applicant did not sponsor the investigational new drug. A predecessor in interest is an entity, e.g., a corporation, that the applicant has taken over, merged with, or purchased, or from which the applicant has purchased all rights to the drug. Purchase of nonexclusive rights to a clinical investigation after it is completed is not sufficient to satisfy this definition.

Date of approval means the date on the letter from FDA stating that the new drug application is approved, whether or not final printed labeling or other materials must yet be submitted as long as approval of such labeling or materials is not expressly required. ''Date of approval'' refers only to a final approval and not to a tentative approval that may become effective at a later date.

Essential to approval means, with regard to an investigation, that there are no other data available that could support approval of the application.

FDA means the Food and Drug Administration.

New chemical entity means a drug that contains no active moiety that has been approved by FDA in any other application submitted under section 505(b) of the act.

New clinical investigation means an investigation in humans the results of which have not been relied on by FDA to demonstrate substantial evidence of effectiveness of a previously approved drug product for any indication or of safety for a new patient population and do not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness or safety in a new patient population of a previously approved drug product. For purposes of this section, data from a clinical investigation previously submitted for use in the comprehensive evaluation of the safety of a drug product but not to support the effectiveness of the drug product would be considered new.

(b) *Submission of and effective date of approval of an abbreviated new drug application submitted under section 505(j) of the act or a 505(b)(2) application.* (1) [Reserved]

(2) If a drug product that contains a new chemical entity was approved after September 24, 1984, in an application submitted under section 505(b) of the act, no person may submit a 505(b)(2) application or abbreviated new drug application under section 505(j) of the act for a drug product that contains the same active moiety as in the new chemical entity for a period of 5 years from the date of approval of the first approved new drug application, except that the 505(b)(2) application or abbreviated application may be submitted after 4 years if it contains a certification of patent invalidity or noninfringement described in § 314.50(i)(1)(i)(A)(*4*) or § 314.94(a)(12)(i)(A)(*4*).

(3) The approval of a 505(b)(2) application or abbreviated application described in paragraph (b)(2) of this section will become effective as provided in § 314.107(b)(1) or (b)(2), unless the

owner of a patent that claims the drug, the patent owner's representative, or exclusive licensee brings suit for patent infringement against the applicant during the 1-year period beginning 48 months after the date of approval of the new drug application for the new chemical entity and within 45 days after receipt of the notice described at § 314.52 or § 314.95, in which case, approval of the 505(b)(2) application or abbreviated application will be made effective as provided in § 314.107(b)(3).

(4) If an application:

(i) Was submitted under section 505(b) of the act;

(ii) Was approved after September 24, 1984;

(iii) Was for a drug product that contains an active moiety that has been previously approved in another application under section 505(b) of the act; and

(iv) Contained reports of new clinical investigations (other than bioavailability studies) conducted or sponsored by the applicant that were essential to approval of the application, the agency will not make effective for a period of 3 years after the date of approval of the application the approval of a 505(b)(2) application or an abbreviated new drug application for the conditions of approval of the original application, or an abbreviated new drug application submitted pursuant to an approved petition under section 505(j)(2)(C) of the act that relies on the information supporting the conditions of approval of an original new drug application.

(5) If a supplemental application:

(i) Was approved after September 24, 1984; and

(ii) Contained reports of new clinical investigations (other than bioavailability studies) that were conducted or sponsored by the applicant that were essential to approval of the supplemental application, the agency will not make effective for a period of 3 years after the date of approval of the supplemental application the approval of a 505(b)(2) application or an abbreviated new drug application for a change, or an abbreviated new drug application submitted pursuant to an approved petition under section 505(j)(2)(C) of the act that relies on the information supporting a change approved in the supplemental new drug application.

[59 FR 50368, Oct. 3, 1994]

§ 314.110 Approvable letter to the applicant.

(a) In selected circumstances, it is useful at the end of the review period for the Food and Drug Administration to indicate to the applicant that the application or abbreviated application is basically approvable providing certain issues are resolved. An approvable letter may be issued in such circumstances. FDA will send the applicant an approvable letter if the application or abbreviated application substantially meets the requirements of this part and the agency believes that it can approve the application or abbreviated application if specific additional information or material is submitted or specific conditions (for example, certain changes in labeling) are agreed to by the applicant. The approvable letter will describe the information or material FDA requires or the conditions the applicant is asked to meet. As a practical matter, the approvable letter will serve in most instances as a mechanism for resolving outstanding issues on drugs that are about to be approved and marketed. For an application or an abbreviated antibiotic application, the applicant shall, within 10 days after the date of the approvable letter:

(1) Amend the application or abbreviated antibiotic application or notify FDA of an intent to file an amendment. The filing of an amendment or notice of intent to file an amendment constitutes an agreement by the applicant to extend the review period for 45 days after the date FDA receives the amendment. The extension is to permit the agency to review the amendment;

(2) Withdraw the application or abbreviated antibiotic application. FDA will consider the applicant's failure to respond within 10 days to an approvable letter to be a request by the applicant to withdraw the application under § 314.65 or the abbreviated antibiotic application under § 314.99. A decision to withdraw an application or abbreviated antibiotic application is without prejudice to a refiling;

(3) For a new drug application or abbreviated antibiotic application, ask the agency to provide the applicant an opportunity for a hearing on the question of whether there are grounds for denying approval of the application under section 505(d) of the act. The applicant shall submit the request to the Associate Director for Policy (HFD–5), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Within 60 days of the date of the approvable letter, or within a different time period to which FDA and the applicant agree, the agency will either approve the application or abbreviated antibiotic application under § 314.105 or refuse to approve the application or abbreviated antibiotic application under § 314.125 and give the applicant written notice of an opportunity for a hearing under § 314.200 and section 505(c)(2) of the act on the question of whether there are grounds for denying approval of the application under section 505(d) of the act;

(4) For an antibiotic, file a petition or notify FDA of an intent to file a petition proposing the issuance, amendment, or repeal of a regulation under § 314.300 and section 507(f) of the act; or

(5) Notify FDA that the applicant agrees to an extension of the review period under section 505(c) of the act, so that the applicant can determine whether to respond further under paragraph (a)(1), (a)(2), (a)(3), or (a)(4) of this section. The applicant's notice is required to state the length of the extension. FDA will honor any reasonable request for such an extension. FDA will consider the applicant's failure to respond further within the extended review period to be a request to withdraw the application under § 314.65 or the abbreviated antibiotic application under § 314.99. A decision to withdraw an application or abbreviated antibiotic application is without prejudice to a refiling.

(b) FDA will send the applicant of an abbreviated new drug application an approvable letter only if the application substantially meets the requirements of this part and the agency believes that it can approve the abbreviated application if minor deficiencies (e.g., labeling deficiencies) are corrected. The approvable letter will describe the deficiencies and state a time period within which the applicant must respond. Unless the applicant corrects the deficiencies by amendment within the specified time period, FDA will refuse to approve the abbreviated application under § 314.127. Within 10 days after the date of the approvable letter, the applicant may also ask the agency to provide the applicant an opportunity for a hearing on the question of whether there are grounds for denying approval of the abbreviated new drug application. Applicants who request a hearing shall submit the request to the Associate Director for Policy (HFD–5), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

[57 FR 17989, Apr. 28, 1992, as amended at 62 FR 43639, Aug. 15, 1997]

§ 314.120 Not approvable letter to the applicant.

(a) The Food and Drug Administration will send the applicant a not approvable letter if the agency believes that the application or abbreviated antibiotic application may not be approved for one of the reasons given in § 314.125 or the abbreviated new drug application may not be approved for one of the reasons given in § 314.127. The not approvable letter will describe the deficiencies in the application or abbreviated application. Except as provided in paragraph (b) of this section, within 10 days after the date of the not approvable letter, the applicant shall:

(1) Amend the application or abbreviated application or notify FDA of an intent to file an amendment. The filing of an amendment or a notice of intent to file an amendment constitutes an agreement by the applicant to extend the review period under § 314.60 or § 314.96;

(2) Withdraw the application or abbreviated application. Except as provided in paragraph (b) of this section, FDA will consider the applicant's failure to respond within 10 days to a not approvable letter to be a request by the applicant to withdraw the application

under § 314.65 or abbreviated application under § 314.99. A decision to withdraw the application or abbreviated application is without prejudice to refiling;

(3) For a new drug application or an abbreviated application, ask the agency to provide the applicant an opportunity for a hearing on the question of whether there are grounds for denying approval of the application under section 505(d) or (j)(3) of the act. The applicant shall submit the request to the Associate Director for Policy (HFD–5), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Within 60 days of the date of the not approvable letter, or within a different time period to which FDA and the applicant agree, the agency will either approve the application or abbreviated application under § 314.105 or refuse to approve the application or abbreviated antibiotic application under § 314.125 or abbreviated new drug application under § 314.127 and give the applicant written notice of an opportunity for a hearing under § 314.200 and section 505(c)(1)(B) or (j)(4)(C) of the act on the question of whether there are grounds for denying approval of the application under section 505(d) or (j)(3) of the act;

(4) For an antibiotic application, file a petition or notify FDA of an intent to file a petition proposing the issuance, amendment, or repeal of a regulation under § 314.300 and section 507(f) of the act; or

(5) Notify FDA that the applicant agrees to an extension of the review period under section 505(c)(1) or (j)(4)(A) of the act, so that the applicant can determine whether to respond further under paragraph (a)(1), (a)(2), (a)(3), or (a)(4) of this section. The applicant's notice is required to state the length of the extension. FDA will honor any reasonable request for such an extension. FDA will consider the applicant's failure to respond further within the extended review period to be a request to withdraw the application under § 314.65 or abbreviated application under § 314.99. A decision to withdraw an application or abbreviated application is without prejudice to a refiling.

(b) With the exception of a request for an opportunity for a hearing under paragraph (a)(3) of this section, the 10-day time period in this section for responding to a not approvable letter does not apply to abbreviated new drug applications. FDA may consider the applicant's failure to respond within 180 days to a not approvable letter to be a request by the applicant to withdraw the abbreviated new drug application under § 314.99.

[57 FR 17990, Apr. 28, 1992, as amended at 62 FR 43639, Aug. 15, 1997]

§ 314.122 Submitting an abbreviated application for, or a 505(j)(2)(C) petition that relies on, a listed drug that is no longer marketed.

(a) An abbreviated new drug application that refers to, or a petition under section 505(j)(2)(C) of the act and § 314.93 that relies on, a listed drug that has been voluntarily withdrawn from sale in the United States must be accompanied by a petition seeking a determination whether the listed drug was withdrawn for safety or effectiveness reasons. The petition must be submitted under §§ 10.25(a) and 10.30 of this chapter and must contain all evidence available to the petitioner concerning the reasons for the withdrawal from sale.

(b) When a petition described in paragraph (a) of this section is submitted, the agency will consider the evidence in the petition and any other evidence before the agency, and determine whether the listed drug is withdrawn from sale for safety or effectiveness reasons, in accordance with the procedures in § 314.161.

(c) An abbreviated new drug application described in paragraph (a) of this section will be disapproved, under § 314.127(a)(11), and a 505(j)(2)(C) petition described in paragraph (a) of this section will be disapproved, under § 314.93(e)(1)(iv), unless the agency determines that the withdrawal of the listed drug was not for safety or effectiveness reasons.

(d) Certain drug products approved for safety and effectiveness that were no longer marketed on September 24, 1984, are not included in the list. Any person who wishes to obtain marketing approval for such a drug product under an abbreviated new drug application must petition FDA for a determination

whether the drug product was withdrawn from the market for safety or effectiveness reasons and request that the list be amended to include the drug product. A person seeking such a determination shall use the petition procedures established in § 10.30 of this chapter. The petitioner shall include in the petition information to show that the drug product was approved for safety and effectiveness and all evidence available to the petitioner concerning the reason that marketing of the drug product ceased.

[57 FR 17990, Apr. 28, 1992; 57 FR 29353, July 1, 1992]

§ 314.125 Refusal to approve an application or abbreviated antibiotic application.

(a) The Food and Drug Administration will refuse to approve the application or abbreviated antibiotic application and for a new drug give the applicant written notice of an opportunity for a hearing under § 314.200 on the question of whether there are grounds for denying approval of the application under section 505(d) of the act, or for an antibiotic publish a proposed regulation based on an acceptable petition under § 314.300, if:

(1) FDA sends the applicant an approvable or a not approvable letter under § 314.110 or § 314.120;

(2) The applicant requests an opportunity for hearing for a new drug on the question of whether the application is approvable or files a petition for an antibiotic proposing the issuance, amendment, or repeal of a regulation; and

(3) FDA finds that any of the reasons given in paragraph (b) of this section apply.

(b) FDA may refuse to approve an application or abbreviated antibiotic application for any of the following reasons:

(1) The methods to be used in, and the facilities and controls used for, the manufacture, processing, packing, or holding of the drug substance or the drug product are inadequate to preserve its identity, strength, quality, purity, stability, and bioavailability.

(2) The investigations required under section 505(b) or 507 of the act do not include adequate tests by all methods reasonably applicable to show whether or not the drug is safe for use under the conditions prescribed, recommended, or suggested in its proposed labeling.

(3) The results of the tests show that the drug is unsafe for use under the conditions prescribed, recommended, or suggested in its proposed labeling or the results do not show that the drug product is safe for use under those conditions.

(4) There is insufficient information about the drug to determine whether the product is safe for use under the conditions prescribed, recommended, or suggested in its proposed labeling.

(5) There is a lack of substantial evidence consisting of adequate and well-controlled investigations, as defined in § 314.126, that the drug product will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in its proposed labeling.

(6) The proposed labeling is false or misleading in any particular.

(7) The application or abbreviated antibiotic application contains an untrue statement of a material fact.

(8) The drug product's proposed labeling does not comply with the requirements for labels and labeling in part 201.

(9) The application or abbreviated antibiotic application does not contain bioavailability or bioequivalence data required under part 320 of this chapter.

(10) A reason given in a letter refusing to file the application or abbreviated antibiotic application under § 314.101(d), if the deficiency is not corrected.

(11) The drug will be manufactured or processed in whole or in part in an establishment that is not registered and not exempt from registration under section 510 of the act and part 207.

(12) The applicant does not permit a properly authorized officer or employee of the Department of Health and Human Services an adequate opportunity to inspect the facilities, controls, and any records relevant to the application or abbreviated antibiotic application.

(13) The methods to be used in, and the facilities and controls used for, the manufacture, processing, packing, or holding of the drug substance or the

drug product do not comply with the current good manufacturing practice regulations in parts 210 and 211.

(14) The application or abbreviated antibiotic application does not contain an explanation of the omission of a report of any investigation of the drug product sponsored by the applicant, or an explanation of the omission of other information about the drug pertinent to an evaluation of the application or abbreviated antibiotic application that is received or otherwise obtained by the applicant from any source.

(15) A nonclinical laboratory study that is described in the application or abbreviated antibiotic application and that is essential to show that the drug is safe for use under the conditions prescribed, recommended, or suggested in its proposed labeling was not conducted in compliance with the good laboratory practice regulations in part 58 of this chapter and no reason for the noncompliance is provided or, if it is, the differences between the practices used in conducting the study and the good laboratory practice regulations do not support the validity of the study.

(16) Any clinical investigation involving human subjects described in the application or abbreviated antibiotic application, subject to the institutional review board regulations in part 58 of this chapter or informed consent regulations in part 50 of this chapter, was not conducted in compliance with those regulations such that the rights or safety of human subjects were not adequately protected.

(17) The applicant or contract research organization that conducted a bioavailability or bioequivalence study described in § 320.38 or § 320.63 of this chapter that is contained in the application or abbreviated antibiotic application refuses to permit an inspection of facilities or records relevant to the study by a properly authorized officer or employee of the Department of Health and Human Services or refuses to submit reserve samples of the drug products used in the study when requested by FDA.

(18) For a new drug, the application failed to contain the patent information required by section 505(b)(1) of the act.

(c) For drugs intended to treat life-threatening or severely-debilitating illnesses that are developed in accordance with §§ 312.80 through 312.88 of this chapter, the criteria contained in paragraphs (b) (3), (4), and (5) of this section shall be applied according to the considerations contained in § 312.84 of this chapter.

[50 FR 7493, Feb. 22, 1985, as amended at 53 FR 41524, Oct. 21, 1988; 57 FR 17991, Apr. 28, 1992; 58 FR 25926, Apr. 28, 1993]

§ 314.126 Adequate and well-controlled studies.

(a) The purpose of conducting clinical investigations of a drug is to distinguish the effect of a drug from other influences, such as spontaneous change in the course of the disease, placebo effect, or biased observation. The characteristics described in paragraph (b) of this section have been developed over a period of years and are recognized by the scientific community as the essentials of an adequate and well-controlled clinical investigation. The Food and Drug Administration considers these characteristics in determining whether an investigation is adequate and well-controlled for purposes of sections 505 and 507 of the act. Reports of adequate and well-controlled investigations provide the primary basis for determining whether there is ''substantial evidence'' to support the claims of effectiveness for new drugs and antibiotics. Therefore, the study report should provide sufficient details of study design, conduct, and analysis to allow critical evaluation and a determination of whether the characteristics of an adequate and well-controlled study are present.

(b) An adequate and well-controlled study has the following characteristics:

(1) There is a clear statement of the objectives of the investigation and a summary of the proposed or actual methods of analysis in the protocol for the study and in the report of its results. In addition, the protocol should contain a description of the proposed methods of analysis, and the study report should contain a description of the methods of analysis ultimately used. If

the protocol does not contain a description of the proposed methods of analysis, the study report should describe how the methods used were selected.

(2) The study uses a design that permits a valid comparison with a control to provide a quantitative assessment of drug effect. The protocol for the study and report of results should describe the study design precisely; for example, duration of treatment periods, whether treatments are parallel, sequential, or crossover, and whether the sample size is predetermined or based upon some interim analysis. Generally, the following types of control are recognized:

(i) *Placebo concurrent control.* The test drug is compared with an inactive preparation designed to resemble the test drug as far as possible. A placebo-controlled study may include additional treatment groups, such as an active treatment control or a dose-comparison control, and usually includes randomization and blinding of patients or investigators, or both.

(ii) *Dose-comparison concurrent control.* At least two doses of the drug are compared. A dose-comparison study may include additional treatment groups, such as placebo control or active control. Dose-comparison trials usually include randomization and blinding of patients or investigators, or both.

(iii) *No treatment concurrent control.* Where objective measurements of effectiveness are available and placebo effect is negligible, the test drug is compared with no treatment. No treatment concurrent control trials usually include randomization.

(iv) *Active treatment concurrent control.* The test drug is compared with known effective therapy; for example, where the condition treated is such that administration of placebo or no treatment would be contrary to the interest of the patient. An active treatment study may include additional treatment groups, however, such as a placebo control or a dose-comparison control. Active treatment trials usually include randomization and blinding of patients or investigators, or both. If the intent of the trial is to show similarity of the test and control drugs, the report of the study should assess the ability of the study to have detected a difference between treatments. Similarity of test drug and active control can mean either that both drugs were effective or that neither was effective. The analysis of the study should explain why the drugs should be considered effective in the study, for example, by reference to results in previous placebo-controlled studies of the active control drug.

(v) *Historical control.* The results of treatment with the test drug are compared with experience historically derived from the adequately documented natural history of the disease or condition, or from the results of active treatment, in comparable patients or populations. Because historical control populations usually cannot be as well assessed with respect to pertinent variables as can concurrent control populations, historical control designs are usually reserved for special circumstances. Examples include studies of diseases with high and predictable mortality (for example, certain malignancies) and studies in which the effect of the drug is self-evident (general anesthetics, drug metabolism).

(3) The method of selection of subjects provides adequate assurance that they have the disease or condition being studied, or evidence of susceptibility and exposure to the condition against which prophylaxis is directed.

(4) The method of assigning patients to treatment and control groups minimizes bias and is intended to assure comparability of the groups with respect to pertinent variables such as age, sex, severity of disease, duration of disease, and use of drugs or therapy other than the test drug. The protocol for the study and the report of its results should describe how subjects were assigned to groups. Ordinarily, in a concurrently controlled study, assignment is by randomization, with or without stratification.

(5) Adequate measures are taken to minimize bias on the part of the subjects, observers, and analysts of the data. The protocol and report of the study should describe the procedures used to accomplish this, such as blinding.

(6) The methods of assessment of subjects' response are well-defined and reliable. The protocol for the study and

the report of results should explain the variables measured, the methods of observation, and criteria used to assess response.

(7) There is an analysis of the results of the study adequate to assess the effects of the drug. The report of the study should describe the results and the analytic methods used to evaluate them, including any appropriate statistical methods. The analysis should assess, among other things, the comparability of test and control groups with respect to pertinent variables, and the effects of any interim data analyses performed.

(c) The Director of the Center for Drug Evaluation and Research may, on the Director's own initiative or on the petition of an interested person, waive in whole or in part any of the criteria in paragraph (b) of this section with respect to a specific clinical investigation, either prior to the investigation or in the evaluation of a completed study. A petition for a waiver is required to set forth clearly and concisely the specific criteria from which waiver is sought, why the criteria are not reasonably applicable to the particular clinical investigation, what alternative procedures, if any, are to be, or have been employed, and what results have been obtained. The petition is also required to state why the clinical investigations so conducted will yield, or have yielded, substantial evidence of effectiveness, notwithstanding nonconformance with the criteria for which waiver is requested.

(d) For an investigation to be considered adequate for approval of a new drug, it is required that the test drug be standardized as to identity, strength, quality, purity, and dosage form to give significance to the results of the investigation.

(e) Uncontrolled studies or partially controlled studies are not acceptable as the sole basis for the approval of claims of effectiveness. Such studies carefully conducted and documented, may provide corroborative support of well-controlled studies regarding efficacy and may yield valuable data regarding safety of the test drug. Such studies will be considered on their merits in the light of the principles listed here, with the exception of the requirement for the comparison of the treated subjects with controls. Isolated case reports, random experience, and reports lacking the details which permit scientific evaluation will not be considered.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11580, Mar. 29, 1990]

§ 314.127 Refusal to approve an abbreviated new drug application.

(a) FDA will refuse to approve an abbreviated application for a new drug under section 505(j) of the act for any of the following reasons:

(1) The methods used in, or the facilities and controls used for, the manufacture, processing, and packing of the drug product are inadequate to ensure and preserve its identity, strength, quality, and purity.

(2) Information submitted with the abbreviated new drug application is insufficient to show that each of the proposed conditions of use has been previously approved for the listed drug referred to in the application.

(3)(i) If the reference listed drug has only one active ingredient, information submitted with the abbreviated new drug application is insufficient to show that the active ingredient is the same as that of the reference listed drug;

(ii) If the reference listed drug has more than one active ingredient, information submitted with the abbreviated new drug application is insufficient to show that the active ingredients are the same as the active ingredients of the reference listed drug; or

(iii) If the reference listed drug has more than one active ingredient and if the abbreviated new drug application is for a drug product that has an active ingredient different from the reference listed drug:

(A) Information submitted with the abbreviated new drug application is insufficient to show:

(*1*) That the other active ingredients are the same as the active ingredients of the reference listed drug; or

(*2*) That the different active ingredient is an active ingredient of a listed

drug or a drug that does not meet the requirements of section 201(p) of the act; or

(B) No petition to submit an abbreviated application for the drug product with the different active ingredient was approved under § 314.93.

(4)(i) If the abbreviated new drug application is for a drug product whose route of administration, dosage form, or strength purports to be the same as that of the listed drug referred to in the abbreviated new drug application, information submitted in the abbreviated new drug application is insufficient to show that the route of administration, dosage form, or strength is the same as that of the reference listed drug; or

(ii) If the abbreviated new drug application is for a drug product whose route of administration, dosage form, or strength is different from that of the listed drug referred to in the application, no petition to submit an abbreviated new drug application for the drug product with the different route of administration, dosage form, or strength was approved under § 314.93.

(5) If the abbreviated new drug application was submitted under the approval of a petition under § 314.93, the abbreviated new drug application did not contain the information required by FDA with respect to the active ingredient, route of administration, dosage form, or strength that is not the same as that of the reference listed drug.

(6)(i) Information submitted in the abbreviated new drug application is insufficient to show that the drug product is bioequivalent to the listed drug referred to in the abbreviated new drug application; or

(ii) If the abbreviated new drug application was submitted under a petition approved under § 314.93, information submitted in the abbreviated new drug application is insufficient to show that the active ingredients of the drug product are of the same pharmacological or therapeutic class as those of the reference listed drug and that the drug product can be expected to have the same therapeutic effect as the reference listed drug when administered to patients for each condition of use approved for the reference listed drug.

(7) Information submitted in the abbreviated new drug application is insufficient to show that the labeling proposed for the drug is the same as the labeling approved for the listed drug referred to in the abbreviated new drug application except for changes required because of differences approved in a petition under § 314.93 or because the drug product and the reference listed drug are produced or distributed by different manufacturers or because aspects of the listed drug's labeling are protected by patent, or by exclusivity, and such differences do not render the proposed drug product less safe or effective than the listed drug for all remaining, nonprotected conditions of use.

(8)(i) Information submitted in the abbreviated new drug application of any other information available to FDA shows that:

(A) The inactive ingredients of the drug product are unsafe for use, as described in paragraph (a)(8)(ii) of this section, under the conditions prescribed, recommended, or suggested in the labeling proposed for the drug product; or

(B) The composition of the drug product is unsafe, as described in paragraph (a)(8)(ii) of this section, under the conditions prescribed, recommended, or suggested in the proposed labeling because of the type or quantity of inactive ingredients included or the manner in which the inactive ingredients are included.

(ii)(A) FDA will consider the inactive ingredients or composition of a drug product unsafe and refuse to approve an abbreviated new drug application under paragraph (a)(8)(i) of this section if, on the basis of information available to the agency, there is a reasonable basis to conclude that one or more of the inactive ingredients of the proposed drug or its composition raises serious questions of safety. From its experience with reviewing inactive ingredients, and from other information available to it, FDA may identify changes in inactive ingredients or composition that may adversely affect a drug product's safety. The inactive ingredients or composition of a proposed drug product will be considered to raise serious questions of safety if the product incorporates one or more of these

changes. Examples of the changes that may raise serious questions of safety include, but are not limited to, the following:

(*1*) A change in an inactive ingredient so that the product does not comply with an official compendium.

(*2*) A change in composition to include an inactive ingredient that has not been previously approved in a drug product for human use by the same route of administration.

(*3*) A change in the composition of a parenteral drug product to include an inactive ingredient that has not been previously approved in a parenteral drug product.

(*4*) A change in composition of a drug product for ophthalmic use to include an inactive ingredient that has not been previously approved in a drug for ophthalmic use.

(*5*) The use of a delivery or a modified release mechanism never before approved for the drug.

(*6*) A change in composition to include a significantly greater content of one or more inactive ingredients than previously used in the drug product.

(*7*) If the drug product is intended for topical administration, a change in the properties of the vehicle or base that might increase absorption of certain potentially toxic active ingredients thereby affecting the safety of the drug product, or a change in the lipophilic properties of a vehicle or base, e.g., a change from an oleaginous to a water soluble vehicle or base.

(B) FDA will consider an inactive ingredient in, or the composition of, a drug product intended for parenteral use to be unsafe and will refuse to approve the abbreviated new drug application unless it contains the same inactive ingredients, other than preservatives, buffers, and antioxidants, in the same concentration as the listed drug, and, if it differs from the listed drug in a preservative, buffer, or antioxidant, the application contains sufficient information to demonstrate that the difference does not affect the safety of the drug product.

(C) FDA will consider an inactive ingredient in, or the composition of, a drug product intended for ophthalmic or otic use unsafe and will refuse to approve the abbreviated new drug application unless it contains the same inactive ingredients, other than preservatives, buffers, substances to adjust tonicity, or thickening agents, in the same concentration as the listed drug, and if it differs from the listed drug in a preservative, buffer, substance to adjust tonicity, or thickening agent, the application contains sufficient information to demonstrate that the difference does not affect the safety of the drug product and the labeling does not claim any therapeutic advantage over or difference from the listed drug.

(9) Approval of the listed drug referred to in the abbreviated new drug application has been withdrawn or suspended for grounds described in § 314.150(a) or FDA has published a notice of opportunity for hearing to withdraw approval of the reference listed drug under § 314.150(a).

(10) Approval of the listed drug referred to in the abbreviated new drug application has been withdrawn under § 314.151 or FDA has proposed to withdraw approval of the reference listed drug under § 314.151(a).

(11) FDA has determined that the reference listed drug has been withdrawn from sale for safety or effectiveness reasons under § 314.161, or the reference listed drug has been voluntarily withdrawn from sale and the agency has not determined whether the withdrawal is for safety or effectiveness reasons, or approval of the reference listed drug has been suspended under § 314.153, or the agency has issued an initial decision proposing to suspend the reference listed drug under § 314.153(a)(1).

(12) The abbreviated new drug application does not meet any other requirement under section 505(j)(2)(A) of the act.

(13) The abbreviated new drug application contains an untrue statement of material fact.

(b) FDA may refuse to approve an abbreviated application for a new drug if the applicant or contract research organization that conducted a bioavailability or bioequivalence study described in § 320.63 of this chapter that is contained in the abbreviated new drug application refuses to permit an

inspection of facilities or records relevant to the study by a properly authorized officer of employee of the Department of Health and Human Services or refuses to submit reserve samples of the drug products used in the study when requested by FDA.

[57 FR 17991, Apr. 28, 1992; 57 FR 29353, July 1, 1992, as amended at 58 FR 25927, Apr. 28, 1993]

§ 314.150 Withdrawal of approval of an application or abbreviated application.

(a) The Food and Drug Administration will notify the applicant, and, if appropriate, all other persons who manufacture or distribute identical, related, or similar drug products as defined in §§ 310.6 and 314.151(a) of this chapter and for a new drug afford an opportunity for a hearing on a proposal to withdraw approval of the application or abbreviated new drug application under section 505(e) of the act and under the procedure in § 314.200, or, for an antibiotic, rescind a certification or release, or amend or repeal a regulation providing for certification under section 507 of the act and under the procedure in § 314.300, if any of the following apply:

(1) The Secretary of Health and Human Services has suspended the approval of the application or abbreviated application for a new drug on a finding that there is an imminent hazard to the public health. FDA will promptly afford the applicant an expedited hearing following summary suspension on a finding of imminent hazard to health.

(2) FDA finds:

(i) That clinical or other experience, tests, or other scientific data show that the drug is unsafe for use under the conditions of use upon the basis of which the application or abbreviated application was approved; or

(ii) That new evidence of clinical experience, not contained in the application or not available to FDA until after the application or abbreviated application was approved, or tests by new methods, or tests by methods not deemed reasonably applicable when the application or abbreviated application was approved, evaluated together with the evidence available when the application or abbreviated application was approved, reveal that the drug is not shown to be safe for use under the conditions of use upon the basis of which the application or abbreviated application was approved; or

(iii) Upon the basis of new information before FDA with respect to the drug, evaluated together with the evidence available when the application or abbreviated application was approved, that there is a lack of substantial evidence from adequate and well-controlled investigations as defined in § 314.126, that the drug will have the effect it is purported or represented to have under the conditions of use prescribed, recommended, or suggested in its labeling; or

(iv) That the application or abbreviated application contains any untrue statement of a material fact; or

(v) That the patent information prescribed by section 505(c) of the act was not submitted within 30 days after the receipt of written notice from FDA specifying the failure to submit such information; or

(b) FDA may notify the applicant, and, if appropriate, all other persons who manufacture or distribute identical, related, or similar drug products as defined in § 310.6, and for a new drug afford an opportunity for a hearing on a proposal to withdraw approval of the application or abbreviated new drug application under section 505(e) of the act and under the procedure in § 314.200, or, for an antibiotic, rescind a certification or release, or amend or repeal a regulation providing for certification under section 507 of the act and the procedure in § 314.300, if the agency finds:

(1) That the applicant has failed to establish a system for maintaining required records, or has repeatedly or deliberately failed to maintain required records or to make required reports under section 505(k) or 507(g) of the act and § 314.80, § 314.81, or § 314.98, or that the applicant has refused to permit access to, or copying or verification of, its records.

(2) That on the basis of new information before FDA, evaluated together with the evidence available when the application or abbreviated application was approved, the methods used in, or

the facilities and controls used for, the manufacture, processing, and packing of the drug are inadequate to ensure and preserve its identity, strength, quality, and purity and were not made adequate within a reasonable time after receipt of written notice from the agency.

(3) That on the basis of new information before FDA, evaluated together with the evidence available when the application or abbreviated application was approved, the labeling of the drug, based on a fair evaluation of all material facts, is false or misleading in any particular, and the labeling was not corrected by the applicant within a reasonable time after receipt of written notice from the agency.

(4) That the applicant has failed to comply with the notice requirements of section 510(j)(2) of the act.

(5) That the applicant has failed to submit bioavailability or bioequivalence data required under part 320 of this chapter.

(6) The application or abbreviated application does not contain an explanation of the omission of a report of any investigation of the drug product sponsored by the applicant, or an explanation of the omission of other information about the drug pertinent to an evaluation of the application or abbreviated application that is received or otherwise obtained by the applicant from any source.

(7) That any nonclinical laboratory study that is described in the application or abbreviated application and that is essential to show that the drug is safe for use under the conditions prescribed, recommended, or suggested in its labeling was not conducted in compliance with the good laboratory practice regulations in part 58 of this chapter and no reason for the noncompliance was provided or, if it was, the differences between the practices used in conducting the study and the good laboratory practice regulations do not support the validity of the study.

(8) Any clinical investigation involving human subjects described in the application or abbreviated application, subject to the institutional review board regulations in part 56 of this chapter or informed consent regulations in part 50 of this chapter, was not conducted in compliance with those regulations such that the rights or safety of human subjects were not adequately protected.

(9) That the applicant or contract research organization that conducted a bioavailability or bioequivalence study described in § 320.38 or § 320.63 of this chapter that is contained in the application or abbreviated application refuses to permit an inspection of facilities or records relevant to the study by a properly authorized officer or employee of the Department of Health and Human Services or refuses to submit reserve samples of the drug products used in the study when requested by FDA.

(10) That the labeling for the drug product that is the subject of the abbreviated new drug application is no longer consistent with that for the listed drug referred to in the abbreviated new drug application, except for differences approved in the abbreviated new drug application or those differences resulting from:

(i) A patent on the listed drug issued after approval of the abbreviated new drug application; or

(ii) Exclusivity accorded to the listed drug after approval of the abbreviated new drug application that do not render the drug product less safe or effective than the listed drug for any remaining, nonprotected condition(s) of use.

(c) FDA will withdraw approval of an application or abbreviated application if the applicant requests its withdrawal because the drug subject to the application or abbreviated application is no longer being marketed, provided none of the conditions listed in paragraphs (a) and (b) of this section applies to the drug. FDA will consider a written request for a withdrawal under this paragraph to be a waiver of an opportunity for hearing otherwise provided for in this section. Withdrawal of approval of an application or abbreviated application under this paragraph is without prejudice to refiling.

(d) FDA may notify an applicant that it believes a potential problem associated with a drug is sufficiently serious that the drug should be removed from the market and may ask the applicant to waive the opportunity for hearing

otherwise provided for under this section, to permit FDA to withdraw approval of the application or abbreviated application for the product, and to remove voluntarily the product from the market. If the applicant agrees, the agency will not make a finding under paragraph (b) of this section, but will withdraw approval of the application or abbreviated application in a notice published in the FEDERAL REGISTER that contains a brief summary of the agency's and the applicant's views of the reasons for withdrawal.

[57 FR 17993, Apr. 28, 1992, as amended at 58 FR 25927, Apr. 28, 1993]

§ 314.151 Withdrawal of approval of an abbreviated new drug application under section 505(j)(5) of the act.

(a) Approval of an abbreviated new drug application approved under § 314.105(d) may be withdrawn when the agency withdraws approval, under § 314.150(a) or under this section, of the approved drug referred to in the abbreviated new drug application. If the agency proposed to withdraw approval of a listed drug under § 314.150(a), the holder of an approved application for the listed drug has a right to notice and opportunity for hearing. The published notice of opportunity for hearing will identify all drug products approved under § 314.105(d) whose applications are subject to withdrawal under this section if the listed drug is withdrawn, and will propose to withdraw such drugs. Holders of approved applications for the identified drug products will be provided notice and an opportunity to respond to the proposed withdrawal of their applications as described in paragraphs (b) and (c) of this section.

(b)(1) The published notice of opportunity for hearing on the withdrawal of the listed drug will serve as notice to holders of identified abbreviated new drug applications of the grounds for the proposed withdrawal.

(2) Holders of applications for drug products identified in the notice of opportunity for hearing may submit written comments on the notice of opportunity for hearing issued on the proposed withdrawal of the listed drug. If an abbreviated new drug application holder submits comments on the notice of opportunity for hearing and a hearing is granted, the abbreviated new drug application holder may participate in the hearing as a nonparty participant as provided for in § 12.89 of this chapter.

(3) Except as provided in paragraphs (c) and (d) of this section, the approval of an abbreviated new drug application for a drug product identified in the notice of opportunity for hearing on the withdrawal of a listed drug will be withdrawn when the agency has completed the withdrawal of approval of the listed drug.

(c)(1) If the holder of an application for a drug identified in the notice of opportunity for hearing has submitted timely comments but does not have an opportunity to participate in a hearing because a hearing is not requested or is settled, the submitted comments will be considered by the agency, which will issue an initial decision. The initial decision will respond to the comments, and contain the agency's decision whether there are grounds to withdraw approval of the listed drug and of the abbreviated new drug applications on which timely comments were submitted. The initial decision will be sent to each abbreviated new drug application holder that has submitted comments.

(2) Abbreviated new drug application holders to whom the initial decision was sent may, within 30 days of the issuance of the initial decision, submit written objections.

(3) The agency may, at its discretion, hold a limited oral hearing to resolve dispositive factual issues that cannot be resolved on the basis of written submissions.

(4) If there are no timely objections to the initial decision, it will become final at the expiration of 30 days.

(5) If timely objections are submitted, they will be reviewed and responded to in a final decision.

(6) The written comments received, the initial decision, the evidence relied on in the comments and in the initial decision, the objections to the initial decision, and, if a limited oral hearing has been held, the transcript of that hearing and any documents submitted therein, shall form the record upon which the agency shall make a final decision.

(7) Except as provided in paragraph (d) of this section, any abbreviated new drug application whose holder submitted comments on the notice of opportunity for hearing shall be withdrawn upon the issuance of a final decision concluding that the listed drug should be withdrawn for grounds as described in § 314.150(a). The final decision shall be in writing and shall constitute final agency action, reviewable in a judicial proceeding.

(8) Documents in the record will be publicly available in accordance with § 10.20(j) of this chapter. Documents available for examination or copying will be placed on public display in the Dockets Management Branch (HFA–305), Food and Drug Administration, room. 1–23, 12420 Parklawn Dr., Rockville, MD 20857, promptly upon receipt in that office.

(d) If the agency determines, based upon information submitted by the holder of an abbreviated new drug application, that the grounds for withdrawal of the listed drug are not applicable to a drug identified in the notice of opportunity for hearing, the final decision will state that the approval of the abbreviated new drug application for such drug is not withdrawn.

[57 FR 17994, Apr. 28, 1992]

§ 314.152 Notice of withdrawal of approval of an application or abbreviated application for a new drug.

If the Food and Drug Administration withdraws approval of an application or abbreviated application for a new drug, FDA will publish a notice in the FEDERAL REGISTER announcing the withdrawal of approval. If the application or abbreviated application was withdrawn for grounds described in § 314.150(a) or § 314.151, the notice will announce the removal of the drug from the list of approved drugs published under section 505(j)(6) of the act and shall satisfy the requirement of § 314.162(b).

[57 FR 17994, Apr. 28, 1992]

§ 314.153 Suspension of approval of an abbreviated new drug application.

(a) *Suspension of approval.* The approval of an abbreviated new drug application approved under § 314.105(d) shall be suspended for the period stated when:

(1) The Secretary of the Department of Health and Human Services, under the imminent hazard authority of section 505(e) of the act or the authority of this paragraph, suspends approval of a listed drug referred to in the abbreviated new drug application, for the period of the suspension;

(2) The agency, in the notice described in paragraph (b) of this section, or in any subsequent written notice given an abbreviated new drug application holder by the agency, concludes that the risk of continued marketing and use of the drug is inappropriate, pending completion of proceedings to withdraw or suspend approval under § 314.151 or paragraph (b) of this section; or

(3) The agency, under the procedures set forth in paragraph (b) of this section, issues a final decision stating the determination that the abbreviated application is suspended because the listed drug on which the approval of the abbreviated new drug application depends has been withdrawn from sale for reasons of safety or effectiveness or has been suspended under paragraph (b) of this section. The suspension will take effect on the date stated in the decision and will remain in effect until the agency determines that the marketing of the drug has resumed or that the withdrawal is not for safety or effectiveness reasons.

(b) *Procedures for suspension of abbreviated new drug applications when a listed drug is voluntarily withdrawn for safety or effectiveness reasons.* (1) If a listed drug is voluntarily withdrawn from sale, and the agency determines that the withdrawal from sale was for reasons of safety or effectiveness, the agency will send each holder of an approved abbreviated new drug application that is subject to suspension as a result of this determination a copy of the agency's initial decision setting forth the reasons for the determination. The initial decision will also be placed on file with the Dockets Management Branch (HFA–305), Food and Drug Administration, room 1–23, 12420 Parklawn Dr., Rockville, MD 20857.

(2) Each abbreviated new drug application holder will have 30 days from

the issuance of the initial decision to present, in writing, comments and information bearing on the initial decision. If no comments or information is received, the initial decision will become final at the expiration of 30 days.

(3) Comments and information received within 30 days of the issuance of the initial decision will be considered by the agency and responded to in a final decision.

(4) The agency may, in its discretion, hold a limited oral hearing to resolve dispositive factual issues that cannot be resolved on the basis of written submissions.

(5) If the final decision affirms the agency's initial decision that the listed drug was withdrawn for reasons of safety or effectiveness, the decision will be published in the FEDERAL REGISTER in compliance with § 314.152, and will, except as provided in paragraph (b)(6) of this section, suspend approval of all abbreviated new drug applications identified under paragraph (b)(1) of this section and remove from the list the listed drug and any drug whose approval was suspended under this paragraph. The notice will satisfy the requirement of § 314.162(b). The agency's final decision and copies of materials on which it relies will also be filed with the Dockets Management Branch (address in paragraph (b)(1) of this section).

(6) If the agency determines in its final decision that the listed drug was withdrawn for reasons of safety or effectiveness but, based upon information submitted by the holder of an abbreviated new drug application, also determines that the reasons for the withdrawal of the listed drug are not relevant to the safety and effectiveness of the drug subject to such abbreviated new drug application, the final decision will state that the approval of such abbreviated new drug application is not suspended.

(7) Documents in the record will be publicly available in accordance with § 10.20(j) of this chapter. Documents available for examination or copying will be placed on public display in the Dockets Management Branch (address in paragraph (b)(1) of this section) promptly upon receipt in that office.

[57 FR 17995, Apr. 28, 1992]

§ 314.160 Approval of an application or abbreviated application for which approval was previously refused, suspended, or withdrawn.

Upon the Food and Drug Administration's own initiative or upon request of an applicant, FDA may, on the basis of new data, approve an application or abbreviated application which it had previously refused, suspended, or withdrawn approval. FDA will publish a notice in the FEDERAL REGISTER announcing the approval.

[57 FR 17995, Apr. 28, 1992]

§ 314.161 Determination of reasons for voluntary withdrawal of a listed drug.

(a) A determination whether a listed drug that has been voluntarily withdrawn from sale was withdrawn for safety or effectiveness reasons may be made by the agency at any time after the drug has been voluntarily withdrawn from sale, but must be made:

(1) Prior to approving an abbreviated new drug application that refers to the listed drug;

(2) Whenever a listed drug is voluntarily withdrawn from sale and abbreviated new drug applications that referred to the listed drug have been approved; and

(3) When a person petitions for such a determination under §§ 10.25(a) and 10.30 of this chapter.

(b) Any person may petition under §§ 10.25(a) and 10.30 of this chapter for a determination whether a listed drug has been voluntarily withdrawn for safety or effectiveness reasons. Any such petition must contain all evidence available to the petitioner concerning the reason that the drug is withdrawn from sale.

(c) If the agency determines that a listed drug is withdrawn from sale for safety or effectiveness reasons, the agency will, except as provided in paragraph (d) of this section, publish a notice of the determination in the FEDERAL REGISTER.

(d) If the agency determines under paragraph (a) of this section that a listed drug is withdrawn from sale for safety and effectiveness reasons and there are approved abbreviated new drug applications that are subject to suspension under section 505(j)(5) of the

act, FDA will initiate a proceeding in accordance with § 314.153(b).

(e) A drug that the agency determines is withdrawn for safety or effectiveness reasons will be removed from the list, under § 314.162. The drug may be relisted if the agency has evidence that marketing of the drug has resumed or that the withdrawal is not for safety or effectiveness reasons. A determination that the drug is not withdrawn for safety or effectiveness reasons may be made at any time after its removal from the list, upon the agency's initiative, or upon the submission of a petition under §§ 10.25(a) and 10.30 of this chapter. If the agency determines that the drug is not withdrawn for safety or effectiveness reasons, the agency shall publish a notice of this determination in the FEDERAL REGISTER. The notice will also announce that the drug is relisted, under § 314.162(c). The notice will also serve to reinstate approval of all suspended abbreviated new drug applications that referred to the listed drug.

[57 FR 17995, Apr. 28, 1992]

§ 314.162 Removal of a drug product from the list.

(a) FDA will remove a previously approved new drug product from the list for the period stated when:

(1) The agency withdraws or suspends approval of a new drug application or an abbreviated new drug application under § 314.150(a) or § 314.151 or under the imminent hazard authority of section 505(e) of the act, for the same period as the withdrawal or suspension of the application; or

(2) The agency, in accordance with the procedures in § 314.153(b) or § 314.161, issues a final decision stating that the listed drug was withdrawn from sale for safety or effectiveness reasons, or suspended under § 314.153(b), until the agency determines that the withdrawal from the market has ceased or is not for safety or effectiveness reasons.

(b) FDA will publish in the FEDERAL REGISTER a notice announcing the removal of a drug from the list.

(c) At the end of the period specified in paragraph (a)(1) or (a)(2) of this section, FDA will relist a drug that has been removed from the list. The agency will publish in the FEDERAL REGISTER a notice announcing the relisting of the drug.

[57 FR 17996, Apr. 28, 1992]

§ 314.170 Adulteration and misbranding of an approved drug.

All drugs, including those the Food and Drug Administration approves, or provides for certification of, under sections 505, 506, and 507 of the act and this part, are subject to the adulteration and misbranding provisions in sections 501, 502, and 503 of the act. FDA is authorized to regulate approved new drugs and approved antibiotic drugs by regulations issued through informal rulemaking under sections 501, 502, and 503 of the act.

Subpart E—Hearing Procedures for New Drugs

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted. Redesignated at 57 FR 17983, Apr. 28, 1992.

§ 314.200 Notice of opportunity for hearing; notice of participation and request for hearing; grant or denial of hearing.

(a) *Notice of opportunity for hearing.* The Director of the Center for Drug Evaluation and Research, Food and Drug Administration, will give the applicant, and all other persons who manufacture or distribute identical, related, or similar drug products as defined in § 310.6 of this chapter, notice and an opportunity for a hearing on the Center's proposal to refuse to approve an application or to withdraw the approval of an application or abbreviated application under section 505(e) of the act. The notice will state the reasons for the action and the proposed grounds for the order.

(1) The notice may be general (that is, simply summarizing in a general way the information resulting in the notice) or specific (that is, either referring to specific requirements in the statute and regulations with which there is a lack of compliance, or providing a detailed description and analysis of the specific facts resulting in the notice).

(2) FDA will publish the notice in the FEDERAL REGISTER and will state that

the applicant, and other persons subject to the notice under § 310.6, who wishes to participate in a hearing, has 30 days after the date of publication of the notice to file a written notice of participation and request for hearing. The applicant, or other persons subject to the notice under § 310.6, who fails to file a written notice of participation and request for hearing within 30 days, waives the opportunity for a hearing.

(3) It is the responsibility of every manufacturer and distributor of a drug product to review every notice of opportunity for a hearing published in the FEDERAL REGISTER to determine whether it covers any drug product that person manufactures or distributes. Any person may request an opinion of the applicability of a notice to a specific product that may be identical, related, or similar to a product listed in a notice by writing to the Division of Drug Labeling Compliance (HFD–310), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. A person shall request an opinion within 30 days of the date of publication of the notice to be eligible for an opportunity for a hearing under the notice. If a person requests an opinion, that person's time for filing an appearance and request for a hearing and supporting studies and analyses begins on the date the person receives the opinion from FDA.

(b) FDA will provide the notice of opportunity for a hearing to applicants and to other persons subject to the notice under § 310.6, as follows:

(1) To any person who has submitted an application or abbreviated application, by delivering the notice in person or by sending it by registered or certified mail to the last address shown in the application or abbreviated application.

(2) To any person who has not submitted an application or abbreviated application but who is subject to the notice under § 310.6 of this chapter, by publication of the notice in the FEDERAL REGISTER.

(c)(1) *Notice of participation and request for a hearing, and submission of studies and comments.* The applicant, or any other person subject to the notice under § 310.6, who wishes to participate in a hearing, shall file with the Dockets Management Branch (HFA–305), Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857, (i) within 30 days after the date of the publication of the notice (or of the date of receipt of an opinion requested under paragraph (a)(3) of this section) a written notice of participation and request for a hearing and (ii) within 60 days after the date of publication of the notice, unless a different period of time is specified in the notice of opportunity for a hearing, the studies on which the person relies to justify a hearing as specified in paragraph (d) of this section. The applicant, or other person, may incorporate by reference the raw data underlying a study if the data were previously submitted to FDA as part of an application, abbreviated application, or other report.

(2) FDA will not consider data or analyses submitted after 60 days in determining whether a hearing is warranted unless they are derived from well-controlled studies begun before the date of the notice of opportunity for hearing and the results of the studies were not available within 60 days after the date of publication of the notice. Nevertheless, FDA may consider other studies on the basis of a showing by the person requesting a hearing of inadvertent omission and hardship. The person requesting a hearing shall list in the request for hearing all studies in progress, the results of which the person intends later to submit in support of the request for a hearing. The person shall submit under paragraph (c)(1)(ii) of this section a copy of the complete protocol, a list of the participating investigators, and a brief status report of the studies.

(3) Any other interested person who is not subject to the notice of opportunity for a hearing may also submit comments on the proposal to withdraw approval of the application or abbreviated application. The comments are requested to be submitted within the time and under the conditions specified in this section.

(d) The person requesting a hearing is required to submit under paragraph (c)(1)(ii) of this section the studies (including all protocols and underlying raw data) on which the person relies to

justify a hearing with respect to the drug product. Except, a person who requests a hearing on the refusal to approve an application is not required to submit additional studies and analyses if the studies upon which the person relies have been submitted in the application and in the format and containing the summaries required under § 314.50.

(1) If the grounds for FDA's proposed action concern the effectiveness of the drug, each request for hearing is required to be supported only by adequate and well-controlled clinical studies meeting all of the precise requirements of § 314.126 and, for combination drug products, § 300.50, or by other studies not meeting those requirements for which a waiver has been previously granted by FDA under § 314.126. Each person requesting a hearing shall submit all adequate and well-controlled clinical studies on the drug product, including any unfavorable analyses, views, or judgments with respect to the studies. No other data, information, or studies may be submitted.

(2) The submission is required to include a factual analysis of all the studies submitted. If the grounds for FDA's proposed action concern the effectiveness of the drug, the analysis is required to specify how each study accords, on a point-by-point basis, with each criterion required for an adequate well-controlled clinical investigation established under § 314.126 and, if the product is a combination drug product, with each of the requirements for a combination drug established in § 300.50, or the study is required to be accompanied by an appropriate waiver previously granted by FDA. If a study concerns a drug or dosage form or condition of use or mode of administration other than the one in question, that fact is required to be clearly stated. Any study conducted on the final marketed form of the drug product is required to be clearly identified.

(3) Each person requesting a hearing shall submit an analysis of the data upon which the person relies, except that the required information relating either to safety or to effectiveness may be omitted if the notice of opportunity for hearing does not raise any issue with respect to that aspect of the drug; information on compliance with § 300.50 may be omitted if the drug product is not a combination drug product. A financial certification or disclosure statement or both as required by part 54 of this chapter must accompany all clinical data submitted. FDA can most efficiently consider submissions made in the following format.

I. Safety data.

A. Animal safety data.

1. Individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

B. Human safety data.

1. Individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination about the safety of each individual active component.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination about the safety of each individual active component.

II. Effectiveness data.

A. Individual active components: Controlled studies, with an analysis showing clearly how each study satisfies, on a point-by-point basis, each of the criteria required by § 314.126.

B. Combinations of individual active components.

1. Controlled studies with an analysis showing clearly how each study satisfies on a point-by-point basis, each of the criteria required by § 314.126.

2. An analysis showing clearly how each requirement of § 300.50 has been satisfied.

III. A summary of the data and views setting forth the medical rationale and purpose for the drug and its ingredients and the scientific basis for the conclusion that the drug and its ingredients have been proven safe and/or effective for the intended use. If there is an absence of controlled studies in the material submitted or the requirements of any element of § 300.50 or § 314.126 have not been fully met, that fact is required to be stated clearly and a waiver obtained under § 314.126 is required to be submitted.

IV. A statement signed by the person responsible for such submission that it includes in full (or incorporates by reference as permitted in § 314.200(c)(2)) all studies and information specified in § 314.200(d).

(WARNING: A willfully false statement is a criminal offense, 18 U.S.C. 1001.)

(e) *Contentions that a drug product is not subject to the new drug requirements.* A notice of opportunity for a hearing encompasses all issues relating to the legal status of each drug product subject to it, including identical, related, and similar drug products as defined in § 310.6. A notice of appearance and request for a hearing under paragraph (c)(1)(i) of this section is required to contain any contention that the product is not a new drug because it is generally recognized as safe and effective within the meaning of section 201(p) of the act, or because it is exempt from part or all of the new drug provisions of the act under the exemption for products marketed before June 25, 1938, contained in section 201(p) of the act or under section 107(c) of the Drug Amendments of 1962, or for any other reason. Each contention is required to be supported by a submission under paragraph (c)(1)(ii) of this section and the Commissioner of Food and Drugs will make an administrative determination on each contention. The failure of any person subject to a notice of opportunity for a hearing, including any person who manufactures or distributes an identical, related, or similar drug product as defined in § 310.6, to submit a notice of participation and request for hearing or to raise all such contentions constitutes a waiver of any contentions not raised.

(1) A contention that a drug product is generally recognized as safe and effective within the meaning of section 201(p) of the act is required to be supported by submission of the same quantity and quality of scientific evidence that is required to obtain approval of an application for the product, unless FDA has waived a requirement for effectiveness (under § 314.126) or safety, or both. The submission should be in the format and with the analyses required under paragraph (d) of this section. A person who fails to submit the required scientific evidence required under paragraph (d) waives the contention. General recognition of safety and effectiveness shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information.

(2) A contention that a drug product is exempt from part or all of the new drug provisions of the act under the exemption for products marketed before June 25, 1938, contained in section 201(p) of the act, or under section 107(c) of the Drug Amendments of 1962, is required to be supported by evidence of past and present quantitative formulas, labeling, and evidence of marketing. A person who makes such a contention should submit the formulas, labeling, and evidence of marketing in the following format.

I. Formulation.

A. A copy of each pertinent document or record to establish the exact quantitative formulation of the drug (both active and inactive ingredients) on the date of initial marketing of the drug.

B. A statement whether such formulation has at any subsequent time been changed in any manner. If any such change has been made, the exact date, nature, and rationale for each change in formulation, including any deletion or change in the concentration of any active ingredient and/or inactive ingredient, should be stated, together with a copy of each pertinent document or record to establish the date and nature of each such change, including, but not limited to, the formula which resulted from each such change. If no such change has been made, a copy of representative documents or records showing the formula at representative points in time should be submitted to support the statement.

II. Labeling.

A. A copy of each pertinent document or record to establish the identity of each item of written, printed, or graphic matter used as labeling on the date the drug was initially marketed.

B. A statement whether such labeling has at any subsequent time been discontinued or changed in any manner. If such discontinuance or change has been made, the exact date, nature, and rationale for each discontinuance or change and a copy of each pertinent document or record to establish each such discontinuance or change should be submitted, including, but not limited to, the labeling which resulted from each such discontinuance or change. If no such discontinuance or change has been made, a copy of representative documents or records showing labeling at representative points in time should be submitted to support the statement.

III. Marketing.

A. A copy of each pertinent document or record to establish the exact date the drug was initially marketed.

B. A statement whether such marketing has at any subsequent time been discontinued. If such marketing has been discontinued, the exact date of each such discontinuance should be submitted, together with a copy of each pertinent document or record to establish each such date.

IV. Verification.

A statement signed by the person responsible for such submission, that all appropriate records have been searched and to the best of that person's knowledge and belief it includes a true and accurate presentation of the facts.

(WARNING: A willfully false statement is a criminal offense, 18 U.S.C. 1001.)

(3) The Food and Drug Administration will not find a drug product, including any active ingredient, which is identical, related, or similar, as described in § 310.6, to a drug product, including any active ingredient for which an application is or at any time has been effective or deemed approved, or approved under section 505 of the act, to be exempt from part or all of the new drug provisions of the act.

(4) A contention that a drug product is not a new drug for any other reason is required to be supported by submission of the factual records, data, and information that are necessary and appropriate to support the contention.

(5) It is the responsibility of every person who manufactures or distributes a drug product in reliance upon a ''grandfather'' provision of the act to maintain files that contain the data and information necessary fully to document and support that status.

(f) *Separation of functions.* Separation of functions commences upon receipt of a request for hearing. The Director of the Center for Drug Evaluation and Research, Food and Drug Administration, will prepare an analysis of the request and a proposed order ruling on the matter. The analysis and proposed order, the request for hearing, and any proposed order denying a hearing and response under paragraph (g) (2) or (3) of this section will be submitted to the Office of the Commissioner of Food and Drugs for review and decision. When the Center for Drug Evaluation and Research recommends denial of a hearing on all issues on which a hearing is requested, no representative of the Center will participate or advise in the review and decision by the Commissioner. When the Center for Drug Evaluation and Research recommends that a hearing be granted on one or more issues on which a hearing is requested, separation of functions terminates as to those issues, and representatives of the Center may participate or advise in the review and decision by the Commissioner on those issues. The Commissioner may modify the text of the issues, but may not deny a hearing on those issues. Separation of functions continues with respect to issues on which the Center for Drug Evaluation and Research has recommended denial of a hearing. The Commissioner will neither evaluate nor rule on the Center's recommendation on such issues and such issues will not be included in the notice of hearing. Participants in the hearing may make a motion to the presiding officer for the inclusion of any such issue in the hearing. The ruling on such a motion is subject to review in accordance with § 12.35(b). Failure to so move constitutes a waiver of the right to a hearing on such an issue. Separation of functions on all issues resumes upon issuance of a notice of hearing. The Office of the General Counsel, Department of Health and Human Services, will observe the same separation of functions.

(g) *Summary judgment.* A person who requests a hearing may not rely upon allegations or denials but is required to set forth specific facts showing that there is a genuine and substantial issue of fact that requires a hearing with respect to a particular drug product specified in the request for hearing.

(1) Where a specific notice of opportunity for hearing (as defined in paragraph (a)(1) of this section) is used, the Commissioner will enter summary judgment against a person who requests a hearing, making findings and conclusions, denying a hearing, if it conclusively appears from the face of the data, information, and factual analyses in the request for the hearing that there is no genuine and substantial issue of fact which precludes the refusal to approve the application or abbreviated application or the withdrawal of approval of the application or

abbreviated application; for example, no adequate and well-controlled clinical investigations meeting each of the precise elements of § 314.126 and, for a combination drug product, § 300.50 of this chapter, showing effectiveness have been identified. Any order entering summary judgment is required to set forth the Commissioner's findings and conclusions in detail and is required to specify why each study submitted fails to meet the requirements of the statute and regulations or why the request for hearing does not raise a genuine and substantial issue of fact.

(2) When following a general notice of opportunity for a hearing (as defined in paragraph (a)(1) of this section) the Director of the Center for Drug Evaluation and Research concludes that summary judgment against a person requesting a hearing should be considered, the Director will serve upon the person requesting a hearing by registered mail a proposed order denying a hearing. This person has 60 days after receipt of the proposed order to respond with sufficient data, information, and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(3) When following a general or specific notice of opportunity for a hearing a person requesting a hearing submits data or information of a type required by the statute and regulations, and the Director of the Center for Drug Evaluation and Research concludes that summary judgment against the person should be considered, the Director will serve upon the person by registered mail a proposed order denying a hearing. The person has 60 days after receipt of the proposed order to respond with sufficient data, information, and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(4) If review of the data, information, and analyses submitted show that the grounds cited in the notice are not valid, for example, that substantial evidence of effectiveness exists, the Commissioner will enter summary judgment for the person requesting the hearing, and rescind the notice of opportunity for hearing.

(5) If the Commissioner grants a hearing, it will begin within 90 days after the expiration of the time for requesting the hearing unless the parties otherwise agree in the case of denial of approval, and as soon as practicable in the case of withdrawal of approval.

(6) The Commissioner will grant a hearing if there exists a genuine and substantial issue of fact or if the Commissioner concludes that a hearing would otherwise be in the public interest.

(7) If the manufacturer or distributor of an identical, related, or similar drug product requests and is granted a hearing, the hearing may consider whether the product is in fact identical, related, or similar to the drug product named in the notice of opportunity for a hearing.

(8) A request for a hearing, and any subsequent grant or denial of a hearing, applies only to the drug products named in such documents.

(h) FDA will issue a notice withdrawing approval and declaring all products unlawful for drug products subject to a notice of opportunity for a hearing, including any identical, related, or similar drug product under § 310.6, for which an opportunity for a hearing is waived or for which a hearing is denied. The Commissioner may defer or stay the action pending a ruling on any related request for a hearing or pending any related hearing or other administrative or judicial proceeding.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11580, Mar. 29, 1990; 57 FR 17996, Apr. 28, 1992; 59 FR 14364, Mar. 28, 1994; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 314.200 was amended in paragraph (d)(3) by adding a new sentence after the first sentence, effective Feb. 2, 1999.

§ 314.201 Procedure for hearings.

Parts 10 through 16 apply to hearings relating to new drugs under section 505 (d) and (e) of the act.

§ 314.235 Judicial review.

(a) The Commissioner of Food and Drugs will certify the transcript and

record. In any case in which the Commissioner enters an order without a hearing under § 314.200(g), the record certified by the Commissioner is required to include the requests for hearing together with the data and information submitted and the Commissioner's findings and conclusion.

(b) A manufacturer or distributor of an identical, related, or similar drug product under § 310.6 may seek judicial review of an order withdrawing approval of a new drug application, whether or not a hearing has been held, in a United States court of appeals under section 505(h) of the act.

Subpart F—Administrative Procedures for Antibiotics

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted. Redesignated at 57 FR 17983, Apr. 28, 1992.

§ 314.300 Procedure for the issuance, amendment, or repeal of regulations.

(a) The procedures in part 10 apply to the issuance, amendment, or repeal of regulations under section 507 of the act.

(b)(1) The Commissioner of Food and Drugs, on his or her own initiative or on the application or request of any interested person, may publish in the FEDERAL REGISTER a notice of proposed rulemaking and order to issue, amend, or repeal any regulation contemplated by section 507 of the act. The notice and order may be general (that is, simply summarizing in a general way the information resulting in the notice and order) or specific (that is, either referring to specific requirements in the statute and regulations with which there is a lack of compliance, or providing a detailed description and analysis of the specific facts resulting in the notice and order).

(2) The Food and Drug Administration will give interested persons an opportunity to submit written comments and to request an informal conference on the proposal, unless the notice and opportunity for comment and informal conference have already been provided in connection with the announcement of the reports of the National Academy of Sciences/National Research Council, Drug Efficacy Study Group, to persons who will be adversely affected, or as provided in §§ 10.40(e) and 12.20(c)(2). A person is required to request an informal conference within 30 days of the notice of proposed rulemaking unless otherwise specified in the notice. If an informal conference is requested and granted, those persons participating in the conference may submit comments, within 30 days of the conference, unless otherwise specified in the proposal.

(3) It is the responsibility of every manufacturer and distributor of an antibiotic drug product to review every proposal published in the FEDERAL REGISTER to determine whether it covers any drug product that person manufactures or distributes.

(4) After considering the written comments, the results of any conference, and the data available, the Commissioner will publish an order in the FEDERAL REGISTER acting on the proposal, with an opportunity for any person who will be adversely affected to file objections, to request a hearing, and to show reasonable grounds for the hearing. Any person who wishes to participate in a hearing, shall file with the Dockets Management Branch (HFA–305), Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857, (i) within 30 days after the date of the publication of the order a written notice of participation and request for a hearing and (ii) within 60 days after the date of publication of the order, unless a different period of time is specified in the order, the studies on which the person relies to justify a hearing as specified in paragraph (b)(6) of this section. The person may incorporate by reference the raw data underlying a study if the data were previously submitted to FDA as part of an application or other report.

(5) FDA will not consider data or analysis submitted after 60 days in determining whether a hearing is warranted unless they are derived from well-controlled studies begun before the date of the order and the results of the studies were not available within 60 days after the date of publication of the order. Nevertheless, FDA may consider other studies on the basis of a showing by the person requesting a hearing of inadvertent omission and

hardship. The person requesting a hearing shall list in the request for hearing all studies in progress, the results of which the person intends later to submit in support of the request for hearing. The person shall submit under paragraph (b)(4)(ii) of this section a copy of the complete protocol, a list of the participating investigators, and a brief status report of the studies.

(6) The person requesting a hearing is required to submit as required under § 314.200(c)(1)(ii) the studies (including all protocols and underlying raw data) on which the person relies to justify a hearing with respect to the drug product. A financial certification or disclosure statement or both as required by part 54 of this chapter must accompany all clinical data submitted with the request for hearing. Except, a person who requests a hearing on a proposal is not required to submit additional studies and analyses if the studies upon which the person relies have been submitted in an application and in the format and containing the summaries required under § 314.50.

(i) If the grounds for FDA proposed action concern the effectiveness of the drug, each request for hearing is required to be supported only by adequate and well-controlled clinical studies meeting all of the precise requirements of § 314.126 and, for combination drug products, § 300.50, or by other studies not meeting those requirements for which a waiver has been previously granted by FDA under § 314.126. Each person requesting a hearing shall submit all adequate and well-controlled clinical studies on the drug product, any unfavorable analyses, views, or judgments with respect to the studies. No other data, information, or studies may be submitted.

(ii) The submission is required to include a factual analysis of all the studies submitted. If the grounds for FDA proposed action concern the effectiveness of the drug, the analysis is required to specify how each study accords, on a point-by-point basis, with each criterion required for an adequate well-controlled clinical investigation established under § 314.126 and, if the product is a combination drug product, with each of the requirements for a combination drug established in § 300.50, or the study is required to be accompanied by an appropriate waiver previously granted by FDA. If a study concerns a drug entity or dosage form or condition of use or mode of administration other than the one in question, that fact is required to be clearly stated. Any study conducted on the final marketed form of the drug product is required to be clearly identified.

(iii) Each person requesting a hearing shall submit an analysis of the data upon which the person relies, except that the required information relating either to safety or to effectiveness may be omitted if the notice of opportunity for hearing does not raise any issue with respect to that aspect of the drug; information on compliance with § 300.50 may be omitted if the drug product is not a combination drug product. FDA can most efficiently consider submissions made in the following format.

I. Safety data.

A. Animal safety data.

1. Individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

B. Human safety data.

1. Individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination about the safety of each individual active component.

2. Combinations of the individual active components.

a. Controlled studies.

b. Partially controlled or uncontrolled studies.

c. Documented case reports.

d. Pertinent marketing experiences that may influence a determination about the safety of each individual active component.

II. Effectiveness data.

A. Individual active components: Controlled studies, with an analysis showing clearly how each study satisfies, on a point-by-point basis, each of the criteria required by § 314.126.

B. Combinations of individual active components.

1. Controlled studies with an analysis showing clearly how each study satisfies on

a point-by-point basis, each of the criteria required by § 314.126.

2. An analysis showing clearly how each requirement of § 300.50 has been satisfied.

III. A summary of the data and views setting forth the medical rationale and purpose for the drug and its ingredients and the scientific basis for the conclusion that the drug and its ingredients have been proven safe and/or effective for the intended use. If there is an absence of controlled studies in the material submitted or the requirements of any element of § 300.50 or § 314.126 have not been fully met, that fact is required to be stated clearly and a waiver obtained under § 314.126 is required to be submitted.

IV. A statement signed by the person responsible for such submission that it includes in full (or incorporates by reference as permitted in § 314.200(c)(2)) all studies and information specified in § 314.200(d).

(WARNING: A willfully false statement is a criminal offense, 18 U.S.C. 1001.)

(7) *Separation of functions.* Separation of functions commences upon receipt of a request for hearing. The Director of the Center for Drug Evaluation and Research will prepare an analysis of the request and a proposed order ruling on the matter. The analysis and proposed order, the request for hearing, and any proposed order denying a hearing and response under paragraph (b)(8) (ii) or (iii) of this section will be submitted to the Office of the Commissioner for review and decision. When the Center for Drug Evaluation and Research recommends denial of a hearing on all issues on which a hearing is requested, no representative of the Center will participate or advise in the review and decision by the Commissioner. When the Center for Drug Evaluation and Research recommends that a hearing be granted on one or more issues on which a hearing is requested, separation of functions terminates as to those issues, and representatives of the Center may participate or advise in the review and decision by the Commissioner on those issues. The Commissioner may modify the text of the issues, but may not deny a hearing on those issues. Separation of functions continues with respect to issues on which the Center for Drug Evaluatioon and Research has recommended denial of a hearing. The Commissioner will neither evaluate nor rule on the Center's recommendation on such issues and such issues will not be included in the notice of hearing. Participants in the hearing may make a motion to the presiding officer for the inclusion of any such issue in the hearing. The ruling on such a motion is subject to review in accordance with § 12.35(b). Failure to so move constitutes a waiver of the right to a hearing on such an issue. Separation of functions on all issues resumes upon issuance of a notice of hearing. The Office of the General Counsel, Department of Health and Human Services, will observe the same separation of functions.

(8) *Summary judgment.* A person who requests a hearing may not rely upon allegations or denials but is required to set forth specific facts showing that there is a genuine and substantial issue of fact that requires a hearing with respect to a particular drug product specified in the request for hearing.

(i) Where a specific notice of opportunity for hearing (as defined in paragraph (b)(1) of this section) is used, the Commissioner will enter summary judgment against a person who requests a hearing, making findings and conclusions, denying a hearing, if it conclusively appears from the face of the data, information, and factual analyses in the request for the hearing that there is no genuine and substantial issue of fact which precludes the refusal to approve the application or the withdrawal of approval of the application; for example, no adequate and well-controlled clinical investigations meeting each of the precise elements of § 314.126 and, for a combination drug product, § 300.50, showing effectiveness have been identified. Any order entering summary judgment is required to set forth the Commissioner's findings and conclusions in detail and is required to specify why each study submitted fails to meet the requirements of the statute and regulations or why the request for hearing does not raise a genuine and substantial issue of fact.

(ii) When following a general notice of opportunity for a hearing (as defined in paragraph (b)(1) of this section) the Director of the Center for Drug Evaluation and Research concludes that summary judgment against a person requesting a hearing should be considered, the Director will serve upon the

person requesting a hearing by registered mail a proposed order denying a hearing. This person has 60 days after receipt of the proposed order to respond with sufficient data, information, and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(iii) When following a general or specific notice of opportunity for a hearing a person requesting a hearing submits data or information of a type required by the statute and regulations, and the Director of the Center for Drug Evaluation and Research concludes that summary judgment against the person should be considered, the Director will serve upon the person by registered mail a proposed order denying a hearing. The person has 60 days after receipt of the proposed order to respond with sufficient data, information, and analyses to demonstrate that there is a genuine and substantial issue of fact which justifies a hearing.

(iv) If review of the data, information, and analyses submitted show that the basis for the order is not valid, for example, that substantial evidence of effectiveness exists, the Commissioner will enter summary judgment for the person requesting the hearing, and revoke the order. If a hearing is not requested, the order will become effective as published.

(v) If the Commissioner grants a hearing, it will be conducted under part 12.

(vi) The Commissioner will grant a hearing if there exists a genuine and substantial issue of fact or if the Commissioner concludes that a hearing would otherwise be in the public interest.

(9) The repeal of any regulation constitutes a revocation of all outstanding certificates based upon such regulation. However, the Commissioner may, in his or her discretion, defer or stay such action pending a ruling on any related request for a hearing or pending any related hearing or other administrative or judicial proceeding.

(c) Whenever any interested person submits an application or request under section 507 of the act and part 314 and FDA sends the person an approvable letter under § 314.110 or a not approvable letter under § 314.120, the person may file a petition proposing the issuance, amendment, or repeal of the regulation under the provisions of section 507(f) of the act and part 10. The Commissioner shall cause the regulation proposed in the petition to be published in the FEDERAL REGISTER within 60 days of the receipt of an acceptable petition and further proceedings shall be in accord with the provisions of sections 507(f) and 701 (f) and (g) of the act and part 10.

(d) (1) FDA will not promulgate a regulation providing for the certification of any batch of any drug composed wholly or in part of any kind of penicillin, streptomycin, chlortetracycline, chloramphenicol, bacitracin, or any other antibiotic drug, or any derivative thereof, intended for human use and no existing regulation will be continued in effect unless it is established by substantial evidence that the drug will have such characteristics of identity, strength, quality, and purity necessary to adequately ensure safety and efficacy of use. ''Substantial evidence'' has been defined by Congress to mean ''evidence consisting of adequate and well-controlled investigations, including clinical investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have the effect it purports or is represented to have under the conditions prescribed, recommended, or suggested in the labeling or proposed labeling thereof.'' This definition is made applicable to a number of antibiotic drugs by section 507(h) of the act and it is the test of efficacy that FDA will apply in promulgating, amending, or repealing regulations for all antibiotics under section 507(a) of the act as well.

(2) The scientific essentials of an adequate and well-controlled clinical investigation are described in § 314.126.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985; 50 FR 14212, Apr. 11, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11580, Mar. 29, 1990; 59 FR 14365, Mar. 28, 1994; 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 314.300 was amended in the introductory text of paragraph (b)(6) by adding a new sentence after the first sentence, effective Feb. 2, 1999.

Subpart G—Miscellaneous Provisions

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted. Redesignated at 57 FR 17983, Apr. 28, 1992.

§ 314.410 Imports and exports of new drugs and antibiotics.

(a) *Imports.* (1) A new drug or an antibiotic may be imported into the United States if: (i) It is the subject of an approved application under this part or, in the case of an antibiotic not exempt from certification under part 433, it is also certified or released; or (ii) it complies with the regulations pertaining to investigational new drugs under part 312; and it complies with the general regulations pertaining to imports under subpart E of part 1.

(2) A drug substance intended for use in the manufacture, processing, or repacking of a new drug may be imported into the United States if it complies with the labeling exemption in § 201.122 pertaining to shipments of drug substances in domestic commerce.

(b) *Exports.* (1) A new drug or an antibiotic may be exported if it is the subject of an approved application under this part, and, in the case of an antibiotic, it is certified or released, or it complies with the regulations pertaining to investigational new drugs under part 312.

(2) A new drug substance that is covered by an application approved under this part for use in the manufacture of an approved drug product may be exported by the applicant or any person listed as a supplier in the approved application, provided the drug substance intended for export meets the specifications of, and is shipped with a copy of the labeling required for, the approved drug product.

(3) An antibiotic drug product or drug substance that is subject to certification under section 507 of the act, but which has not been certified or released, may be exported under section 801(e) of the act if it meets the following conditions:

(i) It meets the specifications of the foreign purchaser;

(ii) It is not in conflict with the laws of the country to which it is intended for export;

(iii) It is labeled on the outside of the shipping package that it is intended for export; and

(iv) It is not sold or offered for sale in the United States.

§ 314.420 Drug master files.

(a) A drug master file is a submission of information to the Food and Drug Administration by a person (the drug master file holder) who intends it to be used for one of the following purposes: To permit the holder to incorporate the information by reference when the holder submits an investigational new drug application under part 312 or submits an application or an abbreviated application or an amendment or supplement to them under this part, or to permit the holder to authorize other persons to rely on the information to support a submission to FDA without the holder having to disclose the information to the person. FDA ordinarily neither independently reviews drug master files nor approves or disapproves submissions to a drug master file. Instead, the agency customarily reviews the information only in the context of an application under part 312 or this part. A drug master file may contain information of the kind required for any submission to the agency, including information about the following:

(1) Manufacturing site, facilities, operating procedures, and personnel (because an FDA on-site inspection of a foreign drug manufacturing facility presents unique problems of planning and travel not presented by an inspection of a domestic manufacturing facility, this information is only recommended for foreign manufacturing establishments);

(2) Drug substance, drug substance intermediate, and materials used in their preparation, or drug product;

(3) Packaging materials;

(4) Excipient, colorant, flavor, essence, or materials used in their preparation;

(5) FDA-accepted reference information. (A person wishing to submit information and supporting data in a drug master file (DMF) that is not covered by Types I through IV DMF's must first submit a letter of intent to the Drug Master File Staff, Food and Drug Administration, 12420 Parklawn Dr., Rm. 2–14, Rockville, MD 20852. FDA will then contact the person to discuss the proposed submission.)

(b) An investigational new drug application or an application, abbreviated application, amendment, or supplement may incorporate by reference all or part of the contents of any drug master file in support of the submission if the holder authorizes the incorporation in writing. Each incorporation by reference is required to describe the incorporated material by name, reference number, volume, and page number of the drug master file.

(c) A drug master file is required to be submitted in two copies. The agency has prepared under § 10.90(b) a guideline that provides information about how to prepare a well-organized drug master file. If the drug master file holder adds, changes, or deletes any information in the file, the holder shall notify in writing, each person authorized to reference that information. Any addition, change, or deletion of information in a drug master file (except the list required under paragraph (d) of this section) is required to be submitted in two copies and to describe by name, reference number, volume, and page number the information affected in the drug master file.

(d) The drug master file is required to contain a complete list of each person currently authorized to incorporate by reference any information in the file, identifying by name, reference number, volume, and page number the information that each person is authorized to incorporate. If the holder restricts the authorization to particular drug products, the list is required to include the name of each drug product and the application number, if known, to which the authorization applies.

(e) The public availability of data and information in a drug master file, including the availability of data and information in the file to a person authorized to reference the file, is determined under part 20 and § 314.430.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0001)

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 53 FR 33122, Aug. 30, 1988; 55 FR 28380, July 11, 1990]

§ 314.430 Availability for public disclosure of data and information in an application or abbreviated application.

(a) The Food and Drug Administration will determine the public availability of any part of an application or abbreviated application under this section and part 20 of this chapter. For purposes of this section, the application or abbreviated application includes all data and information submitted with or incorporated by reference in the application or abbreviated application, including investigational new drug applications, drug master files under § 314.420, supplements submitted under § 314.70 or § 314.97, reports under § 314.80 or § 314.98, and other submissions. For purposes of this section, safety and effectiveness data include all studies and tests of a drug on animals and humans and all studies and tests of the drug for identity, stability, purity, potency, and bioavailability.

(b) FDA will not publicly disclose the existence of an application or abbreviated application before an approvable letter is sent to the applicant under § 314.110, unless the existence of the application or abbreviated application has been previously publicly disclosed or acknowledged. The Center for Drug Evaluation and Research will maintain and make available for public disclosure a list of applications or abbreviated applications for which the agency has sent an approvable letter to the applicant.

(c) If the existence of an unapproved application or abbreviated application has not been publicly disclosed or acknowledged, no data or information in the application or abbreviated application is available for public disclosure.

(d)(1) If the existence of an application or abbreviated application has

been publicly disclosed or acknowledged before the agency sends an approval letter to the applicant, no data or information contained in the application or abbreviated application is available for public disclosure before the agency sends an approval letter, but the Commissioner may, in his or her discretion, disclose a summary of selected portions of the safety and effectiveness data that are appropriate for public consideration of a specific pending issue; for example, for consideration of an open session of an FDA advisory committee.

(2) Notwithstanding paragraph (d)(1) of this section, FDA will make available to the public upon request the information in the investigational new drug application that was required to be filed in Docket Number 95S–0158 in the Dockets Management Branch (HFA–305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1–23, Rockville, MD 20857, for investigations involving an exception from informed consent under § 50.24 of this chapter. Persons wishing to request this information shall submit a request under the Freedom of Information Act.

(e) After FDA sends an approval letter to the applicant, the following data and information in the application or abbreviated application are immediately available for public disclosure, unless the applicant shows that extraordinary circumstances exist. A list of approved applications and abbreviated applications, entitled ''Approved Drug Products with Therapeutic Equivalence Evaluations,'' is available from the Government Printing Office, Washington, DC 20402. This list is updated monthly.

(1) [Reserved]

(2) If the application applies to a new drug, all safety and effectiveness data previously disclosed to the public as set forth in § 20.81 and a summary or summaries of the safety and effectiveness data and information submitted with or incorporated by reference in the application. The summaries do not constitute the full reports of investigations under section 505(b)(1) of the act (21 U.S.C. 355(b)(1)) on which the safety or effectiveness of the drug may be approved. The summaries consist of the following:

(i) For an application approved before July 1, 1975, internal agency records that describe safety and effectiveness data and information, for example, a summary of the basis for approval or internal reviews of the data and information, after deletion of the following:

(*a*) Names and any information that would identify patients or test subjects or investigators.

(*b*) Any inappropriate gratuitous comments unnecessary to an objective analysis of the data and information.

(ii) For an application approved on or after July 1, 1975, a Summary Basis of Approval (SBA) document that contains a summary of the safety and effectiveness data and information evaluated by FDA during the drug approval process. The SBA is prepared in one of the following ways:

(*a*) Before approval of the application, the applicant may prepare a draft SBA which the Center for Drug Evaluation and Research will review and may revise. The draft may be submitted with the application or as an amendment.

(*b*) The Center for Drug Evaluation and Research may prepare the SBA.

(3) A protocol for a test or study, unless it is shown to fall within the exemption established for trade secrets and confidential commercial information in § 20.61.

(4) Adverse reaction reports, product experience reports, consumer complaints, and other similar data and information after deletion of the following:

(i) Names and any information that would identify the person using the product.

(ii) Names and any information that would identify any third party involved with the report, such as a physician or hospital or other institution.

(5) A list of all active ingredients and any inactive ingredients previously disclosed to the public as set forth in § 20.81.

(6) An assay method or other analytical method, unless it serves no regulatory or compliance purpose and is shown to fall within the exemption established for trade secrets and confidential commercial information in § 20.61.

(7) All correspondence and written summaries of oral discussions between FDA and the applicant relating to the application, under the provisions of part 20.

(8) All records showing the testing of an action on a particular lot of a certifiable antibiotic by FDA.

(f) All safety and effectiveness data and information which have been submitted in an application and which have not previously been disclosed to the public are available to the public, upon request, at the time any one of the following events occurs unless extraordinary circumstances are shown:

(1) No work is being or will be undertaken to have the application approved.

(2) A final determination is made that the application is not approvable and all legal appeals have been exhausted.

(3) Approval of the application is withdrawn and all legal appeals have been exhausted.

(4) A final determination has been made that the drug is not a new drug.

(5) For applications submitted under section 505(b) of the act, the effective date of the approval of the first abbreviated application submitted under section 505(j) of the act which refers to such drug, or the date on which the approval of an abbreviated application under section 505(j) of the act which refers to such drug could be made effective if such an abbreviated application had been submitted.

(6) For applications or abbreviated applications submitted under sections 505(j), 506, and 507 of the act, when FDA sends an approval letter to the applicant.

(g) The following data and information in an application or abbreviated application are not available for public disclosure unless they have been previously disclosed to the public as set forth in § 20.81 of this chapter or they relate to a product or ingredient that has been abandoned and they do not represent a trade secret or confidential commercial or financial information under § 20.61 of this chapter:

(1) Manufacturing methods or processes, including quality control procedures.

(2) Production, sales distribution, and similar data and information, except that any compilation of that data and information aggregated and prepared in a way that does not reveal data or information which is not available for public disclosure under this provision is available for public disclosure.

(3) Quantitative or semiquantitative formulas.

(h) The compilations of information specified in § 20.117 are available for public disclosure.

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11580, Mar. 29, 1990; 57 FR 17996, Apr. 28, 1992; 61 FR 51530, Oct. 2, 1996]

§ 314.440 Addresses for applications and abbreviated applications.

(a) Applicants shall send applications, abbreviated applications, and other correspondence relating to matters covered by this part, except for products listed in paragraph (b) of this section, to the Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, and directed to the appropriate office identified below:

(1) Except as provided in paragraph (a)(4) of this section, an application under § 314.50 or § 314.54 submitted for filing should be directed to the Document and Records Section, 12420 Parklawn Dr., Rockville, MD 20852. Applicants may obtain folders for binding applications from the Consolidated Forms and Publications Distribution Center, Washington Commerce Center, 3222 Hubbard Rd., Landover, MD 20785. After FDA has filed the application, the agency will inform the applicant which division is responsible for the application. Amendments, supplements, resubmissions, requests for waivers, and other correspondence about an application that has been filed should be directed to the appropriate division.

(2) Except as provided in paragraph (a)(4) of this section, an abbreviated application under § 314.94, and amendments, supplements, and resubmissions should be directed to the Office of Generic Drugs (HFD–600), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Items sent

by parcel post or overnight courier service should be directed to the Office of Generic Drugs (HFD–600), Center for Drug Evaluation and Research, Food and Drug Administration, Metro Park North II, 7500 Standish Place, rm. 150, Rockville, MD 20855. Correspondence not associated with an application should be addressed specifically to the intended office or division and to the person as follows: Center for Drug Evaluation and Research, Food and Drug Administration, Attn: [*insert name of person*], MPN II, HFD–[*insert mail code of office or division*], 5600 Fishers Lane, Rockville, MD 20857. The mail code for the Office of Generic Drugs is HFD–600, the mail code for the Division of Chemistry is HFD–630, and the mail code for the Division of Bioequivalence is HFD–650.

(3) A request for an opportunity for a hearing under § 314.110 or § 314.120 on the question of whether there are grounds for denying approval of an application, except an application under paragraph (b) of this section, should be directed to the Associate Director for Policy (HFD–5).

(4) The field copy of an application, an abbreviated application, amendments, supplements, resubmissions, requests for waivers, and other correspondence about an application and an abbreviated application shall be sent to the applicant's home FDA district office, except that a foreign applicant shall send the field copy to the appropriate address identified in paragraphs (a)(1) and (a)(2) of this section.

(b) Applicants shall send applications and other correspondence relating to matters covered by this part for the drug products listed below to the Division of Product Certification (HFB–240), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, except applicants shall send a request for an opportunity for a hearing under § 314.110 or § 314.120 on the question of whether there are grounds for denying approval of an application to the Director, Center for Biologics Evaluation and Research (HFB–1), at the same address.

(1) Ingredients packaged together with containers intended for the collection, processing, or storage of blood and blood components.

(2) Urokinase products.

(3) Plasma volume expanders and hydroxyethyl starch for leukapheresis.

[50 FR 7493, Feb. 22, 1985, as amended at 50 FR 21238, May 23, 1985; 55 FR 11581, Mar. 29, 1990; 57 FR 17997, Apr. 28, 1992; 58 FR 47352, Sept. 8, 1993; 62 FR 43639, Aug. 15, 1997]

§ 314.445 Guidelines.

(a) The Food and Drug Administration prepares guidelines under § 10.90(b) to help persons comply with requirements in this part.

(b) The Center for Drug Evaluation and Research will maintain and make publicly available a list of guidelines that apply to the Center's regulations. The list states how a person can obtain a copy of each guideline. A request for a copy of the list should be directed to the CDER Executive Secretariat Staff (HFD–8), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

[50 FR 7493, Feb. 22, 1985, as amended at 55 FR 11581, Mar. 29, 1990; 56 FR 3776, Jan. 31, 1991]

Subpart H—Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses

SOURCE: 57 FR 58958, Dec. 11, 1992, unless otherwise noted.

§ 314.500 Scope.

This subpart applies to certain new drug and antibiotic products that have been studied for their safety and effectiveness in treating serious or life-threatening illnesses and that provide meaningful therapeutic benefit to patients over existing treatments (e.g., ability to treat patients unresponsive to, or intolerant of, available therapy, or improved patient response over available therapy).

§ 314.510 Approval based on a surrogate endpoint or on an effect on a clinical endpoint other than survival or irreversible morbidity.

FDA may grant marketing approval for a new drug product on the basis of adequate and well-controlled clinical

trials establishing that the drug product has an effect on a surrogate endpoint that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit or on the basis of an effect on a clinical endpoint other than survival or irreversible morbidity. Approval under this section will be subject to the requirement that the applicant study the drug further, to verify and describe its clinical benefit, where there is uncertainty as to the relation of the surrogate endpoint to clinical benefit, or of the observed clinical benefit to ultimate outcome. Postmarketing studies would usually be studies already underway. When required to be conducted, such studies must also be adequate and well-controlled. The applicant shall carry out any such studies with due diligence.

§ 314.520 Approval with restrictions to assure safe use.

(a) If FDA concludes that a drug product shown to be effective can be safely used only if distribution or use is restricted, FDA will require such postmarketing restrictions as are needed to assure safe use of the drug product, such as:

(1) Distribution restricted to certain facilities or physicians with special training or experience; or

(2) Distribution conditioned on the performance of specified medical procedures.

(b) The limitations imposed will be commensurate with the specific safety concerns presented by the drug product.

§ 314.530 Withdrawal procedures.

(a) For new drugs and antibiotics approved under §§ 314.510 and 314.520, FDA may withdraw approval, following a hearing as provided in part 15 of this chapter, as modified by this section, if:

(1) A postmarketing clinical study fails to verify clinical benefit;

(2) The applicant fails to perform the required postmarketing study with due diligence;

(3) Use after marketing demonstrates that postmarketing restrictions are inadequate to assure safe use of the drug product;

(4) The applicant fails to adhere to the postmarketing restrictions agreed upon;

(5) The promotional materials are false or misleading; or

(6) Other evidence demonstrates that the drug product is not shown to be safe or effective under its conditions of use.

(b) *Notice of opportunity for a hearing.* The Director of the Center for Drug Evaluation and Research will give the applicant notice of an opportunity for a hearing on the Center's proposal to withdraw the approval of an application approved under § 314.510 or § 314.520. The notice, which will ordinarily be a letter, will state generally the reasons for the action and the proposed grounds for the order.

(c) *Submission of data and information.* (1) If the applicant fails to file a written request for a hearing within 15 days of receipt of the notice, the applicant waives the opportunity for a hearing.

(2) If the applicant files a timely request for a hearing, the agency will publish a notice of hearing in the FEDERAL REGISTER in accordance with §§ 12.32(e) and 15.20 of this chapter.

(3) An applicant who requests a hearing under this section must, within 30 days of receipt of the notice of opportunity for a hearing, submit the data and information upon which the applicant intends to rely at the hearing.

(d) *Separation of functions.* Separation of functions (as specified in § 10.55 of this chapter) will not apply at any point in withdrawal proceedings under this section.

(e) *Procedures for hearings.* Hearings held under this section will be conducted in accordance with the provisions of part 15 of this chapter, with the following modifications:

(1) An advisory committee duly constituted under part 14 of this chapter will be present at the hearing. The committee will be asked to review the issues involved and to provide advice and recommendations to the Commissioner of Food and Drugs.

(2) The presiding officer, the advisory committee members, up to three representatives of the applicant, and up to three representatives of the Center may question any person during or at

the conclusion of the person's presentation. No other person attending the hearing may question a person making a presentation. The presiding officer may, as a matter of discretion, permit questions to be submitted to the presiding officer for response by a person making a presentation.

(f) *Judicial review*. The Commissioner's decision constitutes final agency action from which the applicant may petition for judicial review. Before requesting an order from a court for a stay of action pending review, an applicant must first submit a petition for a stay of action under §10.35 of this chapter.

§314.540 Postmarketing safety reporting.

Drug products approved under this program are subject to the postmarketing recordkeeping and safety reporting applicable to all approved drug products, as provided in §§314.80 and 314.81.

§314.550 Promotional materials.

For drug products being considered for approval under this subpart, unless otherwise informed by the agency, applicants must submit to the agency for consideration during the preapproval review period copies of all promotional materials, including promotional labeling as well as advertisements, intended for dissemination or publication within 120 days following marketing approval. After 120 days following marketing approval, unless otherwise informed by the agency, the applicant must submit promotional materials at least 30 days prior to the intended time of initial dissemination of the labeling or initial publication of the advertisement.

§314.560 Termination of requirements.

If FDA determines after approval that the requirements established in §314.520, §314.530, or §314.550 are no longer necessary for the safe and effective use of a drug product, it will so notify the applicant. Ordinarily, for drug products approved under §314.510, these requirements will no longer apply when FDA determines that the required postmarketing study verifies and describes the drug product's clinical benefit and the drug product would be appropriate for approval under traditional procedures. For drug products approved under §314.520, the restrictions would no longer apply when FDA determines that safe use of the drug product can be assured through appropriate labeling. FDA also retains the discretion to remove specific postapproval requirements upon review of a petition submitted by the sponsor in accordance with §10.30.

PART 316—ORPHAN DRUGS

Subpart A—General Provisions

Sec.
316.1 Scope of this part.
316.2 Purpose.
316.3 Definitions.
316.4 Address for submissions.

Subpart B—Written Recommendations for Investigations of Orphan Drugs

316.10 Content and format of a request for written recommendations.
316.12 Providing written recommendations.
316.14 Refusal to provide written recommendations.

Subpart C—Designation of an Orphan Drug

316.20 Content and format of a request for orphan-drug designation.
316.21 Verification of orphan-drug status.
316.22 Permanent-resident agent for foreign sponsor.
316.23 Timing of requests for orphan-drug designation; designation of already approved drugs.
316.24 Granting orphan-drug designation.
316.25 Refusal to grant orphan-drug designation.
316.26 Amendment to orphan-drug designation.
316.27 Change in ownership of orphan-drug designation.
316.28 Publication of orphan-drug designations.
316.29 Revocation of orphan-drug designation.
316.30 Annual reports of holder of orphan-drug designation.

Subpart D—Orphan-drug Exclusive Approval

316.31 Scope of orphan-drug exclusive approval.
316.34 FDA recognition of exclusive approval.
316.36 Insufficient quantities of orphan drugs.

Subpart E—Open Protocols for Investigations

316.40 Treatment use of a designated orphan drug.

Subpart F—Availability of Information

316.50 Guidelines.
316.52 Availability for public disclosure of data and information in requests and applications.

AUTHORITY: 21 U.S.C. 360aa, 360bb, 360cc, 360dd, 371.

SOURCE: 57 FR 62085, Dec. 29, 1992, unless otherwise noted.

Subpart A—General Provisions

§ 316.1 Scope of this part.

(a) This part implements sections 525, 526, 527, and 528 of the act and provides procedures to encourage and facilitate the development of drugs for rare diseases or conditions, including biological products and antibiotics. This part sets forth the procedures and requirements for:

(1) Submissions to FDA of:

(i) Requests for recommendations for investigations of drugs for rare diseases or conditions;

(ii) Requests for designation of a drug for a rare disease or condition; and

(iii) Requests for gaining exclusive approval for a drug product for a rare disease or condition.

(2) Allowing a sponsor to provide an investigational drug product under a treatment protocol to patients who need the drug for treatment of a rare disease or condition.

(b) This part does not apply to food, medical devices, or drugs for veterinary use.

(c) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

§ 316.2 Purpose.

The purpose of this part is to establish standards and procedures for determining eligibility for the benefits provided for in section 2 of the Orphan Drug Act, including written recommendations for investigations of orphan drugs, a 7-year period of exclusive marketing, and treatment use of investigational orphan drugs. This part is also intended to satisfy Congress' requirements that FDA promulgate procedures for the implementation of sections 525(a) and 526(a) of the act.

§ 316.3 Definitions.

(a) The definitions and interpretations contained in section 201 of the act apply to those terms when used in this part.

(b) The following definitions of terms apply to this part:

(1) *Act* means the Federal Food, Drug, and Cosmetic Act as amended by section 2 of the Orphan Drug Act (sections 525–528 (21 U.S.C. 360aa–360dd)).

(2) *Active moiety* means the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance.

(3) *Clinically superior* means that a drug is shown to provide a significant therapeutic advantage over and above that provided by an approved orphan drug (that is otherwise the same drug) in one or more of the following ways:

(i) Greater effectiveness than an approved orphan drug (as assessed by effect on a clinically meaningful endpoint in adequate and well controlled clinical trials). Generally, this would represent the same kind of evidence needed to support a comparative effectiveness claim for two different drugs; in most cases, direct comparative clinical trials would be necessary; or

(ii) Greater safety in a substantial portion of the target populations, for example, by the elimination of an ingredient or contaminant that is associated with relatively frequent adverse effects. In some cases, direct comparative clinical trials will be necessary; or

(iii) In unusual cases, where neither greater safety nor greater effectiveness has been shown, a demonstration that the drug otherwise makes a major contribution to patient care.

(4) *Director* means the Director of FDA's Office of Orphan Products Development.

(5) *FDA* means the Food and Drug Administration.

(6) *Holder* means the sponsor in whose name an orphan drug is designated and approved.

(7) *IND* means an investigational new drug application under part 312 of this chapter.

(8) *Manufacturer* means any person or agency engaged in the manufacture of a drug that is subject to investigation and approval under the act or the biologics provisions of the Public Health Service Act (42 U.S.C. 262–263).

(9) *Marketing application* means an application for approval of a new drug filed under section 505(b) of the act, a request for certification of an antibiotic under section 507 of the act, or an application for a biological product/establishment license submitted under section 351 of the Public Health Service Act (42 U.S.C. 262).

(10) *Orphan drug* means a drug intended for use in a rare disease or condition as defined in section 526 of the act.

(11) *Orphan-drug designation* means FDA's act of granting a request for designation under section 526 of the act.

(12) *Orphan-drug exclusive approval* or *exclusive approval* means that, effective on the date of FDA approval as stated in the approval letter of a marketing application for a sponsor of a designated orphan drug, no approval will be given to a subsequent sponsor of the same drug product for the same indication for 7 years, except as otherwise provided by law or in this part.

(13) *Same drug* means:

(i) If it is a drug composed of small molecules, a drug that contains the same active moiety as a previously approved drug and is intended for the same use as the previously approved drug, even if the particular ester or salt (including a salt with hydrogen or coordination bonds) or other noncovalent derivative such as a complex, chelate or clathrate has not been previously approved, except that if the subsequent drug can be shown to be clinically superior to the first drug, it will not be considered to be the same drug.

(ii) If it is a drug composed of large molecules (macromolecules), a drug that contains the same principal molecular structural features (but not necessarily all of the same structural features) and is intended for the same use as a previously approved drug, except that, if the subsequent drug can be shown to be clinically superior, it will not be considered to be the same drug. This criterion will be applied as follows to different kinds of macromolecules:

(A) Two protein drugs would be considered the same if the only differences in structure between them were due to post-translational events or infidelity of translation or transcription or were minor differences in amino acid sequence; other potentially important differences, such as different glycosylation patterns or different tertiary structures, would not cause the drugs to be considered different unless the differences were shown to be clinically superior.

(B) Two polysaccharide drugs would be considered the same if they had identical saccharide repeating units, even if the number of units were to vary and even if there were postpolymerization modifications, unless the subsequent drug could be shown to be clinically superior.

(C) Two polynucleotide drugs consisting of two or more distinct nucleotides would be considered the same if they had an identical sequence of purine and pyrimidine bases (or their derivatives) bound to an identical sugar backbone (ribose, deoxyribose, or modifications of these sugars), unless the subsequent drug were shown to be clinically superior.

(D) Closely related, complex partly definable drugs with similar therapeutic intent, such as two live viral vaccines for the same indication, would be considered the same unless the subsequent drug was shown to be clinically superior.

(14) *Sponsor* means the entity that assumes responsibility for a clinical or nonclinical investigation of a drug, including the responsibility for compliance with applicable provisions of the act and regulations. A sponsor may be an individual, partnership, corporation, or Government agency and may be a manufacturer, scientific institution, or an investigator regularly and lawfully engaged in the investigation of drugs. For purposes of the Orphan Drug Act, FDA considers the real party or parties in interest to be a sponsor.

§ 316.4 Address for submissions.

All correspondence and requests for FDA action pursuant to the provisions of this rule should be addressed as follows: Office of Orphan Products Development (HF–35), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

Subpart B—Written Recommendations for Investigations of Orphan Drugs

§ 316.10 Content and format of a request for written recommendations.

(a) A sponsor's request for written recommendations from FDA concerning the nonclinical and clinical investigations necessary for approval of a marketing application shall be submitted in the form and contain the information required in this section. FDA may require the sponsor to submit information in addition to that specified in paragraph (b) of this section if FDA determines that the sponsor's initial request does not contain adequate information on which to base recommendations.

(b) A sponsor shall submit two copies of a completed, dated, and signed request for written recommendations that contains the following:

(1) The sponsor's name and address.

(2) A statement that the sponsor is requesting written recommendations on orphan-drug development under section 525 of the act.

(3) The name of the sponsor's primary contact person and/or resident agent, and the person's title, address, and telephone number.

(4) The generic name and trade name, if any, of the drug and a list of the drug product's components or description of the drug product's formulation, and chemical and physical properties.

(5) The proposed dosage form and route of administration.

(6) A description of the disease or condition for which the drug is proposed to be investigated and the proposed indication or indications for use for such disease or condition.

(7) Current regulatory and marketing status and history of the drug product, including:

(i) Whether the product is the subject of an IND or a marketing application (if the product is the subject of an IND or a marketing application, the IND or marketing application numbers should be stated and the investigational or approved indication or indications for use specified);

(ii) Known marketing experience or investigational status outside the United States;

(iii) So far as is known or can be determined, all indications previously or currently under investigation anywhere;

(iv) All adverse regulatory actions taken by the United States or foreign authorities.

(8) The basis for concluding that the drug is for a disease or condition that is rare in the United States, including the following:

(i) The size and other known demographic characteristics of the patient population affected and the source of this information.

(ii) For drugs intended for diseases or conditions affecting 200,000 or more people in the United States, or for a vaccine, diagnostic drug, or preventive drug that would be given to 200,000 or more persons per year, a summary of the sponsor's basis for believing that the disease or condition described in paragraph (b)(6) of this section occurs so infrequently that there is no reasonable expectation that the costs of drug development and marketing will be recovered in future sales of the drug in the United States. The estimated costs and sales data should be submitted as provided for in § 316.21(c).

(9) A summary and analysis of available data on the pharmacologic effects of the drug.

(10) A summary and analysis of available nonclinical and clinical data pertinent to the drug and the disease to be studied including copies of pertinent published reports. When a drug proposed for orphan drug designation is intended to treat a life-threatening or severely debilitating illness, especially where no satisfactory alternative therapy exists, the sponsor may wish voluntarily to provide this information. A sponsor of such a drug may be entitled

to expeditious development, evaluation, and marketing under 21 CFR part 312, subpart E.

(11) An explanation of how the data summarized and analyzed under paragraphs (b)(9) and (b)(10) of this section support the rationale for use of the drug in the rare disease or condition.

(12) A definition of the population from which subjects will be identified for clinical trials, if known.

(13) A detailed outline of any protocols under which the drug has been or is being studied for the rare disease or condition and a summary and analysis of any available data from such studies.

(14) The sponsor's proposal as to the scope of nonclinical and clinical investigations needed to establish the safety and effectiveness of the drug.

(15) Detailed protocols for each proposed United States or foreign clinical investigation, if available.

(16) Specific questions to be addressed by FDA in its recommendations for nonclinical laboratory studies and clinical investigations.

[57 FR 62085, Dec. 29, 1992; 58 FR 6167, Jan. 26, 1993]

§ 316.12 Providing written recommendations.

(a) FDA will provide the sponsor with written recommendations concerning the nonclinical laboratory studies and clinical investigations necessary for approval of a marketing application if none of the reasons described in § 316.14 for refusing to do so applies.

(b) When a sponsor seeks written recommendations at a stage of drug development at which advice on any clinical investigations, or on particular investigations would be premature, FDA's response may be limited to written recommendations concerning only nonclinical laboratory studies, or only certain of the clinical studies (e.g., Phase 1 studies as described in § 312.21 of this chapter). Prior to providing written recommendations for the clinical investigations required to achieve marketing approval, FDA may require that the results of the nonclinical laboratory studies or completed early clinical studies be submitted to FDA for agency review.

§ 316.14 Refusal to provide written recommendations.

(a) FDA may refuse to provide written recommendations concerning the nonclinical laboratory studies and clinical investigations necessary for approval of a marketing application for any of the following reasons:

(1) The information required to be submitted by § 316.10(b) has not been submitted, or the information submitted is incomplete.

(2) There is insufficient information about:

(i) The drug to identify the active moiety and its physical and chemical properties, if these characteristics can be determined; or

(ii) The disease or condition to determine that the disease or condition is rare in the United States; or

(iii) The reasons for believing that the drug may be useful for treating the rare disease or condition with that drug; or

(iv) The regulatory and marketing history of the drug to determine the scope and type of investigations that have already been conducted on the drug for the rare disease or condition; or

(v) The plan of study for establishing the safety and effectiveness of the drug for treatment of the rare disease or condition.

(3) The specific questions for which the sponsor seeks the advice of the agency are unclear or are not sufficiently specific.

(4) On the basis of the information submitted and on other information available to the agency, FDA determines that the disease or condition for which the drug is intended is not rare in the United States.

(5) On the basis of the information submitted and on other information available to the agency, FDA determines that there is an inadequate basis for permitting investigational use of the drug under part 312 of this chapter for the rare disease or condition.

(6) The request for information contains an untrue statement of material fact.

(b) A refusal to provide written recommendations will be in writing and will include a statement of the reason for FDA's refusal. Where practicable,

FDA will describe the information or material it requires or the conditions the sponsor must meet for FDA to provide recommendations.

(c) Within 90 days after the date of a letter from FDA requesting additional information or material or setting forth the conditions that the sponsor is asked to meet, the sponsor shall either:

(1) Provide the information or material or amend the request for written recommendations to meet the conditions sought by FDA; or

(2) Withdraw the request for written recommendations. FDA will consider a sponsor's failure to respond within 90 days to an FDA letter requesting information or material or setting forth conditions to be met to be a withdrawal of the request for written recommendations.

Subpart C—Designation of an Orphan Drug

§ 316.20 Content and format of a request for orphan-drug designation.

(a) A sponsor that submits a request for orphan-drug designation of a drug for a specified rare disease or condition shall submit each request in the form and containing the information required in paragraph (b) of this section. A sponsor may request orphan-drug designation of a previously unapproved drug, or of a new orphan indication for an already marketed drug. In addition, a sponsor of a drug that is otherwise the same drug as an already approved orphan drug may seek and obtain orphan-drug designation for the subsequent drug for the same rare disease or condition if it can present a plausible hypothesis that its drug may be clinically superior to the first drug. More than one sponsor may receive orphan-drug designation of the same drug for the same rare disease or condition, but each sponsor seeking orphan-drug designation must file a complete request for designation as provided in paragraph (b) of this section.

(b) A sponsor shall submit two copies of a completed, dated, and signed request for designation that contains the following:

(1) A statement that the sponsor requests orphan-drug designation for a rare disease or condition, which shall be identified with specificity.

(2) The name and address of the sponsor; the name of the sponsor's primary contact person and/or resident agent including title, address, and telephone number; the generic and trade name, if any, of the drug or drug product; and the name and address of the source of the drug if it is not manufactured by the sponsor.

(3) A description of the rare disease or condition for which the drug is being or will be investigated, the proposed indication or indications for use of the drug, and the reasons why such therapy is needed.

(4) A description of the drug and a discussion of the scientific rationale for the use of the drug for the rare disease or condition, including all data from nonclinical laboratory studies, clinical investigations, and other relevant data that are available to the sponsor, whether positive, negative, or inconclusive. Copies of pertinent unpublished and published papers are also required.

(5) Where the sponsor of a drug that is otherwise the same drug as an already-approved orphan drug seeks orphan-drug designation for the subsequent drug for the same rare disease or condition, an explanation of why the proposed variation may be clinically superior to the first drug.

(6) Where a drug is under development for only a subset of persons with a particular disease or condition, a demonstration that the subset is medically plausible.

(7) A summary of the regulatory status and marketing history of the drug in the United States and in foreign countries, e.g., IND and marketing application status and dispositions, what uses are under investigation and in what countries; for what indication is the drug approved in foreign countries; what adverse regulatory actions have been taken against the drug in any country.

(8) Documentation, with appended authoritative references, to demonstrate that:

(i) The disease or condition for which the drug is intended affects fewer than 200,000 people in the United States or, if the drug is a vaccine, diagnostic

drug, or preventive drug, the persons to whom the drug will be administered in the United States are fewer than 200,000 per year as specified in § 316.21(b), or

(ii) For a drug intended for diseases or conditions affecting 200,000 or more people, or for a vaccine, diagnostic drug, or preventive drug to be administered to 200,000 or more persons per year in the United States, there is no reasonable expectation that costs of research and development of the drug for the indication can be recovered by sales of the drug in the United States as specified in § 316.21(c).

(9) A statement as to whether the sponsor submitting the request is the real party in interest of the development and the intended or actual production and sales of the product.

(c) Any of the information previously provided by the sponsor to FDA under subpart B of this part may be referenced by specific page or location if it duplicates information required elsewhere in this section.

§ 316.21 Verification of orphan-drug status.

(a) So that FDA can determine whether a drug qualifies for orphan-drug designation under section 526(a) of the act, the sponsor shall include in its request to FDA for orphan-drug designation under § 316.20 either:

(1) Documentation as described in paragraph (b) of this section that the number of people affected by the disease or condition for which the drug product is indicated is fewer than 200,000 persons; or

(2) Documentation as described in paragraph (c) of this section that demonstrates that there is no reasonable expectation that the sales of the drug will be sufficient to offset the costs of developing the drug for the U.S. market and the costs of making the drug available in the United States.

(b) For the purpose of documenting that the number of people affected by the disease or condition for which the drug product is indicated is less than 200,000 persons, ''prevalence'' is defined as the number of persons in the United States who have been diagnosed as having the disease or condition at the time of the submission of the request for orphan-drug designation. To document the number of persons in the United States who have the disease or condition for which the drug is to be indicated, the sponsor shall submit to FDA evidence showing:

(1) The estimated prevalence of the disease or condition for which the drug is being developed, together with a list of the sources (including dates of information provided and literature citations) for the estimate;

(2) Upon request by FDA, the estimated prevalence of any other disease or condition for which the drug has already been approved or for which the drug is currently being developed, together with an explanation of the bases of these estimates; and

(3) The estimated number of people to whom the drug will be administered annually if the drug is a vaccine or is a drug intended for diagnosis or prevention of a rare disease or condition, together with an explanation of the bases of these estimates (including dates of information provided and literature citations).

(c) When submitting documentation that there is no reasonable expectation that costs of research and development of the drug for the disease or condition can be recovered by sales of the drug in the United States, the sponsor shall submit to FDA:

(1) Data on all costs that the sponsor has incurred in the course of developing the drug for the U.S. market. These costs shall include, but are not limited to, nonclinical laboratory studies, clinical studies, dosage form development, record and report maintenance, meetings with FDA, determination of patentability, preparation of designation request, IND/marketing application preparation, distribution of the drug under a ''treatment'' protocol, licensing costs, liability insurance, and overhead and depreciation. Furthermore, the sponsor shall demonstrate the reasonableness of the cost data. For example, if the sponsor has incurred costs for clinical investigations, the sponsor shall provide information on the number of investigations, the years in which they took place, and on the scope, duration, and number of patients that were involved in each investigation.

(2) If the drug was developed wholly or in part outside the United States, in addition to the documentation listed in paragraph (c)(1) of this section:

(i) Data on and justification for all costs that the sponsor has incurred outside of the United States in the course of developing the drug for the U.S. market. The justification, in addition to demonstrating the reasonableness of the cost data, must also explain the method that was used to determine which portion of the foreign development costs should be applied to the U.S. market, and what percent these costs are of total worldwide development costs. Any data submitted to foreign government authorities to support drug pricing determinations must be included with this information.

(ii) Data that show which foreign development costs were recovered through cost recovery procedures that are allowed during drug development in some foreign countries. For example, if the sponsor charged patients for the drug during clinical investigations, the revenues collected by the sponsor must be reported to FDA.

(3) In cases where the drug has already been approved for marketing for any indication or in cases where the drug is currently under investigation for one or more other indications (in addition to the indication for which orphan-drug designation is being sought), a clear explanation of and justification for the method that is used to apportion the development costs among the various indications.

(4) A statement of and justification for any development costs that the sponsor expects to incur after the submission of the designation request. In cases where the extent of these future development costs are not clear, the sponsor should request FDA's advice and assistance in estimating the scope of nonclinical laboratory studies and clinical investigations and other data that are needed to support marketing approval. Based on these recommendations, a cost estimate should be prepared.

(5) A statement of and justification for production and marketing costs that the sponsor has incurred in the past and expects to incur during the first 7 years that the drug is marketed.

(6) An estimate of and justification for the expected revenues from sales of the drug in the United States during its first 7 years of marketing. The justification should assume that the total market for the drug is equal to the prevalence of the disease or condition that the drug will be used to treat. The justification should include:

(i) An estimate of the expected market share of the drug in each of the first 7 years that it is marketed, together with an explanation of the basis for that estimate;

(ii) A projection of and justification for the price at which the drug will be sold; and

(iii) Comparisons with sales of similarly situated drugs, where available.

(7) The name of each country where the drug has already been approved for marketing for any indication, the dates of approval, the indication for which the drug is approved, and the annual sales and number of prescriptions in each country since the first approval date.

(8) A report of an independent certified public accountant in accordance with Statement on Standards for Attestation established by the American Institute of Certified Public Accountants on agreed upon procedures performed with respect to the data estimates and justifications submitted pursuant to this section. Cost data shall be determined in accordance with generally accepted accounting principles.

(d) A sponsor that is requesting orphan-drug designation for a drug designed to treat a disease or condition that affects 200,000 or more persons shall, at FDA's request, allow FDA or FDA-designated personnel to examine at reasonable times and in a reasonable manner all relevant financial records and sales data of the sponsor and manufacturer.

§ 316.22 Permanent-resident agent for foreign sponsor.

Every foreign sponsor that seeks orphan-drug designation shall name a permanent resident of the United States as the sponsor's agent upon whom service of all processes, notices, orders, decisions, requirements, and other communications may be made on

behalf of the sponsor. Notifications of changes in such agents or changes of address of agents should preferably be provided in advance, but not later than 60 days after the effective date of such changes. The permanent-resident agent may be an individual, firm, or domestic corporation and may represent any number of sponsors. The name of the permanent-resident agent shall be provided to: Office of Orphan Products Development (HF–35), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

§ 316.23 Timing of requests for orphan-drug designation; designation of already approved drugs.

(a) A sponsor may request orphan-drug designation at any time in the drug development process prior to the submission of a marketing application for the drug product for the orphan indication.

(b) A sponsor may request orphan-drug designation of an already approved drug product for an unapproved use without regard to whether the prior marketing approval was for an orphan-drug indication.

§ 316.24 Granting orphan-drug designation.

(a) FDA will grant the request for orphan-drug designation if none of the reasons described in § 316.25 for requiring or permitting refusal to grant such a request applies.

(b) When a request for orphan-drug designation is granted, FDA will notify the sponsor in writing and will publicize the orphan-drug designation in accordance with § 316.28.

§ 316.25 Refusal to grant orphan-drug designation.

(a) FDA will refuse to grant a request for orphan-drug designation if any of the following reasons apply:

(1) The drug is not intended for a rare disease or condition because:

(i) There is insufficient evidence to support the estimate that the drug is intended for treatment of a disease or condition in fewer than 200,000 people in the United States, or that the drug is intended for use in prevention or in diagnosis in fewer than 200,000 people annually in the United States; or

(ii) Where the drug is intended for prevention, diagnosis, or treatment of a disease or condition affecting 200,000 or more people in the United States, the sponsor has failed to demonstrate that there is no reasonable expectation that development and production costs will be recovered from sales of the drug for the orphan indication in the United States. A sponsor's failure to comply with § 316.21 shall constitute a failure to make the demonstration required in this paragraph.

(2) There is insufficient information about the drug, or the disease or condition for which it is intended, to establish a medically plausible basis for expecting the drug to be effective in the prevention, diagnosis, or treatment of that disease or condition.

(3) A drug that is otherwise the same drug as one that already has orphan-drug exclusive approval for the same rare disease or condition and the sponsor has not submitted a medically plausible hypothesis for the possible clinical superiority of the subsequent drug.

(b) FDA may refuse to grant a request for orphan-drug designation if the request for designation contains an untrue statement of material fact or omits material information.

§ 316.26 Amendment to orphan-drug designation.

(a) At any time prior to approval of a marketing application for a designated orphan drug, the sponsor holding designation may apply for an amendment to the indication stated in the orphan-drug designation if the proposed change is due to new and unexpected findings in research on the drugs, information arising from FDA recommendations, or unforeseen developments in treatment or diagnosis of the disease or condition.

(b) FDA will grant the amendment if it finds that the initial designation request was made in good faith and that the amendment is intended to conform the orphan-drug designation indication to the results of unanticipated research findings, to unforeseen developments in the treatment or diagnosis of the disease or condition, or to changes based on FDA recommendations, and that, as of the date of the submission

of the amendment request, the amendment would not result in exceeding the prevalence or cost recovery thresholds in § 316.21 (a)(1) or (a)(2) upon which the drug was originally designated.

§ 316.27 Change in ownership of orphan-drug designation.

(a) A sponsor may transfer ownership of or any beneficial interest in the orphan-drug designation of a drug to a new sponsor. At the time of the transfer, the new and former owners are required to submit the following information to FDA:

(1) The former owner or assignor of rights shall submit a letter or other document that states that all or some rights to the orphan-drug designation of the drug have been transferred to the new owner or assignee and that a complete copy of the request for orphan-drug designation, including any amendments to the request, supplements to the granted request, and correspondence relevant to the orphan-drug designation, has been provided to the new owner or assignee.

(2) The new owner or assignee of rights shall submit a statement accepting orphan-drug designation and a letter or other document containing the following:

(i) The date that the change in ownership or assignment of rights is effective;

(ii) A statement that the new owner has a complete copy of the request for orphan-drug designation including any amendments to the request, supplements to the granted request, and correspondence relevant to the orphan-drug designation; and

(iii) A specific description of the rights that have been assigned and those that have been reserved. This may be satisfied by the submission of either a list of rights assigned and reserved or copies of all relevant agreements between assignors and assignees; and

(iv) The name and address of a new primary contact person or resident agent.

(b) No sponsor may relieve itself of responsibilities under the Orphan Drug Act or under this part by assigning rights to another person without:

(1) Assuring that the sponsor or the assignee will carry out such responsibilities; or

(2) Obtaining prior permission from FDA.

[57 FR 62085, Dec. 29, 1992; 58 FR 6167, Jan. 26, 1993]

§ 316.28 Publication of orphan-drug designations.

Each month FDA will update a publically available list of drugs designated as orphan drugs. A cumulative, updated list of all designated drugs will be provided annually. These will be placed on file at the FDA Dockets Management Branch, and will contain the following information:

(a) The name and address of the manufacturer and sponsor;

(b) The generic name and trade name, if any, of the drug and the date of the granting of orphan-drug designation;

(c) The rare disease or condition for which orphan-drug designation was granted; and

(d) The proposed indication for use of the drug.

§ 316.29 Revocation of orphan-drug designation.

(a) FDA may revoke orphan-drug designation for any drug if the agency finds that:

(1) The request for designation contained an untrue statement of material fact; or

(2) The request for designation omitted material information required by this part; or

(3) FDA subsequently finds that the drug in fact had not been eligible for orphan-drug designation at the time of submission of the request therefor.

(b) For an approved drug, revocation of orphan-drug designation also suspends or withdraws the sponsor's exclusive marketing rights for the drug but not the approval of the drug's marketing application.

(c) Where a drug has been designated as an orphan drug because the prevalence of a disease or condition (or, in the case of vaccines, diagnostic drugs, or preventive drugs, the target population) is under 200,000 in the United States at the time of designation, its designation will not be revoked on the

ground that the prevalence of the disease or condition (or the target population) becomes more than 200,000 persons.

§ 316.30 Annual reports of holder of orphan-drug designation.

Within 14 months after the date on which a drug was designated as an orphan drug and annually thereafter until marketing approval, the sponsor of a designated drug shall submit a brief progress report to the FDA Office of Orphan Products Development on the drug that includes:

(a) A short account of the progress of drug development including a review of preclinical and clinical studies initiated, ongoing, and completed and a short summary of the status or results of such studies.

(b) A description of the investigational plan for the coming year, as well as any anticipated difficulties in development, testing, and marketing; and

(c) A brief discussion of any changes that may affect the orphan-drug status of the product. For example, for products nearing the end of the approval process, sponsors should discuss any disparity between the probable marketing indication and the designated indication as related to the need for an amendment to the orphan-drug designation pursuant to § 316.26.

Subpart D—Orphan-drug Exclusive Approval

§ 316.31 Scope of orphan-drug exclusive approval.

(a) After approval of a sponsor's marketing application for a designated orphan-drug product for treatment of the rare disease or condition concerning which orphan-drug designation was granted, FDA will not approve another sponsor's marketing application for the same drug before the expiration of 7 years from the date of such approval as stated in the approval letter from FDA, except that such a marketing application can be approved sooner if, and such time as, any of the following occurs:

(1) Withdrawal of exclusive approval or revocation of orphan-drug designation by FDA under any provision of this part; or

(2) Withdrawal for any reason of the marketing application for the drug in question; or

(3) Consent by the holder of exclusive approval to permit another marketing application to gain approval; or

(4) Failure of the holder of exclusive approval to assure a sufficient quantity of the drug under section 527 of the act and § 316.36.

(b) If a sponsor's marketing application for a drug product is determined not to be approvable because approval is barred under section 527 of the act until the expiration of the period of exclusive marketing of another drug product, FDA will so notify the sponsor in writing.

§ 316.34 FDA recognition of exclusive approval.

(a) FDA will send the sponsor (or, the permanent-resident agent, if applicable) timely written notice recognizing exclusive approval once the marketing application for a designated orphan-drug product has been approved. The written notice will inform the sponsor of the requirements for maintaining orphan-drug exclusive approval for the full 7-year term of exclusive approval.

(b) When a marketing application is approved for a designated orphan drug that qualifies for exclusive approval, FDA will publish in its publication entitled ''Approved Drug Products with Therapeutic Equivalence Evaluations'' information identifying the sponsor, the drug, and the date of termination of the orphan-drug exclusive approval. A subscription to this publication and its monthly cumulative supplements is available from the Superintendent of Documents, Government Printing Office, Washington, DC 20402–9325.

§ 316.36 Insufficient quantities of orphan drugs.

(a) Under section 527 of the act, whenever the Director has reason to believe that the holder of exclusive approval cannot assure the availability of sufficient quantities of an orphan drug to meet the needs of patients with the disease or condition for which the drug was designated, the Director will so notify the holder of this possible insufficiency and will offer the holder one of the following options, which must be

exercised by a time that the Director specifies:

(1) Provide the Director in writing, or orally, or both, at the Director's discretion, views and data as to how the holder can assure the availability of sufficient quantities of the orphan drug within a reasonable time to meet the needs of patients with the disease or condition for which the drug was designated; or

(2) Provide the Director in writing the holder's consent for the approval of other marketing applications for the same drug before the expiration of the 7-year period of exclusive approval.

(b) If, within the time that the Director specifies, the holder fails to consent to the approval of other marketing applications and if the Director finds that the holder has not shown that it can assure the availability of sufficient quantities of the orphan drug to meet the needs of patients with the disease or condition for which the drug was designated, the Director will issue a written order withdrawing the drug product's exclusive approval. This order will embody the Director's findings and conclusions and will constitute final agency action. An order withdrawing the sponsor's exclusive marketing rights may issue whether or not there are other sponsors that can assure the availability of alternative sources of supply. Once withdrawn under this section, exclusive approval may not be reinstated for that drug.

Subpart E—Open Protocols for Investigations

§ 316.40 Treatment use of a designated orphan drug.

Prospective investigators seeking to obtain treatment use of designated orphan drugs may do so as provided in § 312.34 of this chapter.

Subpart F—Availability of Information

§ 316.50 Guidelines.

FDA's Office of Orphan Products Development will maintain and make publicly available a list of guidelines that apply to the regulations in this part. The list states how a person can obtain a copy of each guideline. A request for a copy of the list or for any guideline should be directed to the Office of Orphan Products Development (HF–35), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

§ 316.52 Availability for public disclosure of data and information in requests and applications.

(a) FDA will not publicly disclose the existence of a request for orphan-drug designation under section 526 of the act prior to final FDA action on the request unless the existence of the request has been previously publicly disclosed or acknowledged.

(b) Whether or not the existence of a pending request for designation has been publicly disclosed or acknowledged, no data or information in the request are available for public disclosure prior to final FDA action on the request.

(c) Upon final FDA action on a request for designation, FDA will determine the public availability of data and information in the request in accordance with part 20 and § 314.430 of this chapter and other applicable statutes and regulations.

(d) In accordance with § 316.28, FDA will make a cumulative list of all orphan drug designations available to the public and update such list monthly.

(e) FDA will not publicly disclose the existence of a pending marketing application for a designated orphan drug for the use for which the drug was designated unless the existence of the application has been previously publicly disclosed or acknowledged.

(f) FDA will determine the public availability of data and information contained in pending and approved marketing applications for a designated orphan drug for the use for which the drug was designated in accordance with part 20 and § 314.430 of this chapter and other applicable statutes and regulations.

PART 320—BIOAVAILABILITY AND BIOEQUIVALENCE REQUIREMENTS

Subpart A—General Provisions

Sec.

320.1 Definitions.

Subpart B—Procedures for Determining the Bioavailability or Bioequivalence of Drug Products

320.21 Requirements for submission of in vivo bioavailability and bioequivalence data.
320.22 Criteria for waiver of evidence of in vivo bioavailability or bioequivalence.
320.23 Basis for demonstrating in vivo bioavailability or bioequivalence.
320.24 Types of evidence to establish bioavailability or bioequivalence.
320.25 Guidelines for the conduct of an in vivo bioavailability study.
320.26 Guidelines on the design of a single-dose in vivo bioavailability study.
320.27 Guidelines on the design of a multiple-dose in vivo bioavailability study.
320.28 Correlation of bioavailability with an acute pharmacological effect or clinical evidence.
320.29 Analytical methods for an in vivo bioavailability study.
320.30 Inquiries regarding bioavailability and bioequivalence requirements and review of protocols by the Food and Drug Administration.
320.31 Applicability of requirements regarding an ''Investigational New Drug Application.''
320.32 Procedures for establishing or amending a bioequivalence requirement.
320.33 Criteria and evidence to assess actual or potential bioequivalence problems.
320.34 Requirements for batch testing and certification by the Food and Drug Administration.
320.35 Requirements for in vitro testing of each batch.
320.36 Requirements for maintenance of records of bioequivalence testing.
320.38 Retention of bioavailability samples.
320.63 Retention of bioequivalence samples.

AUTHORITY: 21 U.S.C. 321, 351, 352, 355, 357, 371.

Subpart A—General Provisions

§ 320.1 Definitions.

(a) *Bioavailability* means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

(b) *Drug product* means a finished dosage form, e.g., tablet, capsule, or solution, that contains the active drug ingredient, generally, but not necessarily, in association with inactive ingredients.

(c) *Pharmaceutical equivalents* means drug products that contain identical amounts of the identical active drug ingredient, i.e., the same sat or ester of the same therapeutic moiety, in identical dosage forms, but not necessarily containing the same inactive ingredients, and that meet the identical compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

(d) *Pharmaceutical alternatives* means drug products that contain the identical therapeutic moiety, or its precursor, but not necessarily in the same amount or dosage form or as the same salt or ester. Each such drug product individually meets either the identical or its own respective compendial or other applicable standard of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times and/or dissolution rates.

(e) *Bioequivalence* means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Where there is an intentional difference in rate (e.g., in certain controlled release dosage forms), certain pharmaceutical equivalents or alternatives may be considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug.

(f) *Bioequivalence requirement* means a requirement imposed by the Food and

Drug Administration for in vitro and/or in vivo testing of specified drug products which must be satisfied as a condition of marketing.

[42 FR 1634, Jan. 7, 1977, as amended at 42 FR 1648, Jan. 7, 1977; 57 FR 17997, Apr. 28, 1992]

Subpart B—Procedures for Determining the Bioavailability or Bioequivalence of Drug Products

SOURCE: 42 FR 1648, Jan. 7, 1977, unless otherwise noted.

§ 320.21 Requirements for submission of in vivo bioavailability and bioequivalence data.

(a) Any person submitting a full new drug application to the Food and Drug Administration (FDA) shall include in the application either:

(1) Evidence demonstrating the in vivo bioavailability of the drug product that is the subject of the application; or

(2) Information to permit FDA to waive the submission of evidence demonstrating in vivo bioavailability.

(b) Any person submitting an abbreviated new drug application to FDA shall include in the application either:

(1) Evidence demonstrating that the drug product that is the subject of the abbreviated new drug application is bioequivalent to the reference listed drug (defined in § 314.3(b)); or

(2) Information to show that the drug product is bioequivalent to the reference listed drug which would permit FDA to waive the submission of evidence demonstrating bioequivalence as provided in paragraph (f) of this section.

(c) Any person submitting a supplemental application to FDA shall include in the supplemental application the evidence or information set forth in paragraphs (a) and (b) of this section if the supplemental application proposes any of the following changes:

(1) A change in the manufacturing process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved application.

(2) A change in the labeling to provide for a new indication for use of the drug product, if clinical studies are required to support the new indication for use.

(3) A change in the labeling to provide for a new dosage regimen or for an additional dosage regimen for a special patient population, e.g., infants, if clinical studies are required to support the new or additional dosage regimen.

(d) FDA may approve a full new drug application, or a supplemental application proposing any of the changes set forth in paragraph (c) of this section, that does not contain evidence of in vivo bioavailability or information to permit waiver of the requirement for in vivo bioavailability data, if all of the following conditions are met.

(1) The application was under review by FDA on July 7, 1977.

(2) The application is otherwise approvable.

(3) The application agrees to submit, within the time specified by FDA, either:

(i) Evidence demonstrating the in vivo bioavailability of the drug product that is the subject of the application; or

(ii) Information to permit FDA to waive demonstration of in vivo bioavailability.

(e) Evidence demonstrating the in vivo bioavailability and bioequivalence of a drug product shall be obtained using one of the approaches for determining bioavailability set forth in § 320.24.

(f) Information to permit FDA to waive the submission of evidence demonstrating the in vivo bioavailability or bioequivalence shall meet the criteria set forth in § 320.24.

(g) Any person holding an approved full or abbreviated new drug application shall submit to FDA a supplemental application containing new evidence demonstrating the in vivo bioavailability or bioequivalence of the drug product that is the subject of the application if notified by FDA that:

(1) There are data demonstrating that the dosage regimen in the labeling is based on incorrect assumptions or facts regarding the pharmacokinetics of the drug product and that following this dosage regimen could potentially result in subtherapeutic or toxic levels; or

(2) There are data demonstrating significant intra-batch and batch-to-batch variability, e.g., plus or minus 25 percent, in the bioavailability of the drug product.

(h) The requirements of this section regarding the submission of evidence demonstrating in vivo bioavailability and bioequivalence apply only to a full or abbreviated new drug application or a supplemental application for a finished dosage formulation.

[57 FR 17998, Apr. 28, 1992]

§ 320.22 Criteria for waiver of evidence of in vivo bioavailability or bioequivalence.

(a) Any person submitting a full or abbreviated new drug application, or a supplemental application proposing any of the changes set forth in § 320.21(c), may request FDA to waive the requirement for the submission of evidence demonstrating the in vivo bioavailability or bioequivalence of the drug product that is the subject of the application. An applicant shall submit a request for waiver with the application. Except as provided in paragraph (g) of this section, FDA shall waive the requirement for the submission of evidence of in vivo bioavailability or bioequivalence if the drug product meets any of the provisions of paragraphs (b), (c), (d), or (e) of this section.

(b) For certain drug products, the in vivo bioavailability or bioequivalence of the drug product may be self-evident. FDA shall waive the requirement for the submission of evidence obtained in vivo demonstrating the bioavailability or bioequivalence of these drug products. A drug product's in vivo bioavailability or bioequivalence may be considered self-evident based on other data in the application if the product meets one of the following criteria:

(1) The drug product:

(i) Is a parenteral solution intended solely for administration by injection, or an ophthalmic or otic solution; and

(ii) Contains the same active and inactive ingredients in the same concentration as a drug product that is the subject of an approved full new drug application.

(2) The drug product:

(i) Is administered by inhalation as a gas, e.g., a medicinal or an inhalation anesthetic; and

(ii) Contains an active ingredient in the same dosage form as a drug product that is the subject of an approved full new drug application.

(3) The drug product:

(i) Is a solution for application to the skin, an oral solution, elixir, syrup, tincture, or similar other solubilized form.

(ii) Contains an active drug ingredient in the same concentration and dosage form as a drug product that is the subject of an approved full new drug application; and

(iii) Contains no inactive ingredient or other change in formulation from the drug product that is the subject of the approved full new drug application that may significantly affect absorption of the active drug ingredient or active moiety.

(c) FDA shall waive the requirement for the submission of evidence demonstrating the in vivo bioavailability of a solid oral dosage form (other than an enteric coated or controlled release dosage form) of a drug product determined to be effective for at least one indication in a Drug Efficacy Study Implementation notice or which is identical, related, or similar to such a drug product under § 310.6 of this chapter unless FDA has evaluated the drug product under the criteria set forth in § 320.32, included the drug product in the Approved Drug Products with Therapeutic Equivalence Evaluations List, and rated the drug product as having a known or potential bioequivalence problem. A drug product so rated reflects a determination by FDA that an in vivo bioequivalence study is required.

(d) For certain drug products, bioavailability or bioequivalence may be demonstrated by evidence obtained in vitro in lieu of in vivo data. FDA shall waive the requirement for the submission of evidence obtained in vivo demonstrating the bioavailability of the drug product if the drug product meets one of the following criteria:

(1) [Reserved]

(2) The drug product is in the same dosage form, but in a different strength, and is proportionally similar

in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and the conditions in paragraphs (d)(2)(i) through (d)(2)(iii) of this section are met:

(i) The bioavailability of this other drug product has been demonstrated;

(ii) Both drug products meet an appropriate in vitro test approved by FDA; and

(iii) The applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients.

(iv) This subparagraph does not apply to enteric coated or controlled release dosage forms.

(3) The drug product is, on the basis of scientific evidence submitted in the application, shown to meet an in vitro test that has been correlated with in vivo data.

(4) The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the bioavailability of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met:

(i) The bioavailability of the other product has been demonstrated; and

(ii) Both drug products meet an appropriate in vitro test approved by FDA.

(e) FDA, for good cause, may waive a requirement for the submission of evidence of in vivo bioavailability if waiver is compatible with the protection of the public health. For full new drug applications, FDA may defer a requirement for the submission of evidence of in vivo bioavailability if deferral is compatible with the protection of the public health.

(f) FDA, for good cause, may require evidence of in vivo bioavailability or bioequivalence for any drug product if the agency determines that any difference between the drug product and a listed drug may affect the bioavailability or bioequivalence of the drug product.

[57 FR 17998, Apr. 28, 1992]

§ 320.23 Basis for demonstrating in vivo bioavailability or bioequivalence.

(a)(1) The in vivo bioavailability of a drug product is demonstrated if the product's rate and extent of absorption, as determined by comparison of measured parameters, e.g., concentration of the active drug ingredient in the blood, urinary excretion rates, or pharmacological effects, do not indicate a significant difference from the reference material's rate and extent of absorption. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

(2) Statistical techniques used shall be of sufficient sensitivity to detect differences in rate and extent of absorption that are not attributable to subject variability.

(3) A drug product that differs from the reference material in its rate of absorption, but not in its extent of absorption, may be considered to be bioavailable if the difference in the rate of absorption is intentional, is appropriately reflected in the labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug product.

(b) Two drug products will be considered bioequivalent drug products if they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose. Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on chronic use, and are considered medically insignificant for the particular drug product studied.

[57 FR 17999, Apr. 28, 1992]

§ 320.24 Types of evidence to establish bioavailability or bioequivalence.

(a) Bioavailability or bioequivalence may be determined by several in vivo and in vitro methods. FDA may require in vivo or in vitro testing, or both, to establish the bioavailability of a drug product or the bioequivalence of specific drug products. Information on bioequivalence requirements for specific products is included in the current edition of FDA's publication ''Approved Drug Products with Therapeutic Equivalence Evaluations'' and any current supplement to the publication. The selection of the method used to meet an in vivo or in vitro testing requirement depends upon the purpose of the study, the analytical methods available, and the nature of the drug product. Applicants shall conduct bioavailability and bioequivalence testing using the most accurate, sensitive, and reproducible approach available among those set forth in paragraph (b) of this section. The method used must be capable of demonstrating bioavailability or bioequivalence, as appropriate, for the product being tested.

(b) The following in vivo and in vitro approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the bioavailability or bioequivalence of a drug product.

(1)(i) An in vivo test in humans in which the concentration of the active ingredient or active moiety, and, when appropriate, its active metabolite(s), in whole blood, plasma, serum, or other appropriate biological fluid is measured as a function of time. This approach is particularly applicable to dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution within the body; or

(ii) An in vitro test that has been correlated with and is predictive of human in vivo bioavailability data; or

(iii) An in vivo test in animals that has been correlated with and is predictive of human bioavailability data.

(2) An in vivo test in humans in which the urinary excretion of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time. The intervals at which measurements are taken should ordinarily be as short as possible so that the measure of the rate of elimination is as accurate as possible. Depending on the nature of the drug product, this approach may be applicable to the category of dosage forms described in paragraph (b)(1)(i) of this section. This method is not appropriate where urinary excretion is not a significant mechanism of elimination.

(3) An in vivo test in humans in which an appropriate acute pharmacological effect of the active moiety, and, when appropriate, its active metabolite(s), are measured as a function of time if such effect can be measured with sufficient accuracy, sensitivity, and reproducibility. This approach is applicable to the category of dosage forms described in paragraph (b)(1)(i) of this section only when appropriate methods are not available for measurement of the concentration of the moiety, and, when appropriate, its active metabolite(s), in biological fluids or excretory products but a method is available for the measurement of an appropriate acute pharmacological effect. This approach may be particularly applicable to dosage forms that are not intended to deliver the active moiety to the bloodstream for systemic distribution.

(4) Well-controlled clinical trials in humans that establish the safety and effectiveness of the drug product, for purposes of establishing bioavailability, or appropriately designed comparative clinical trials, for purposes of demonstrating bioequivalence. This approach is the least accurate, sensitive, and reproducible of the general approaches for determining bioavailability or bioequivalence. For dosage forms intended to deliver the active moiety to the bloodstream for systemic distribution, this approach may be considered acceptable only when analytical methods cannot be developed to permit use of one of the approaches outlined in paragraphs (b)(1)(i) and (b)(2) of this section, when the approaches described in paragraphs (b)(1)(ii), (b)(1)(iii), and (b)(3) of this section are not available. This approach may also be considered sufficiently accurate for determining the bioavailability or bioequivalence of dosage forms intended to deliver the

active moiety locally, e.g., topical preparations for the skin, eye, and mucous membranes; oral dosage forms not intended to be absorbed, e.g., an antacid or radiopaque medium; and bronchodilators administered by inhalation if the onset and duration of pharmacological activity are defined.

(5) A currently available in vitro test acceptable to FDA (unusually a dissolution rate test) that ensures human in vivo bioavailability.

(6) Any other approach deemed adequate by FDA to establish bioavailability or bioequivalence.

(c) FDA may, notwithstanding prior requirements for establishing bioavailability or bioequivalence, require in vivo testing in humans of a product at any time if the agency has evidence that the product:

(1) May not produce therapeutic effects comparable to a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably;

(2) May not be bioequivalent to a pharmaceutical equivalent or alternative with which it is intended to be used interchangeably; or

(3) Has greater than anticipated potential toxicity related to pharmacokinetic or other characteristics.

[57 FR 17999, Apr. 28, 1992; 57 FR 29354, July 1, 1992]

§ 320.25 Guidelines for the conduct of an in vivo bioavailability study.

(a) *Guiding principles.* (1) The basic principle in an in vivo bioavailability study is that no unnecessary human research should be done.

(2) An in vivo bioavailability study shall not be conducted in humans if an appropriate animal model exists and correlation of results in animals and humans has been demonstrated. If an appropriate animal model does not exist, however, an in vivo bioavailability study shall ordinarily be done in normal adults under standardized conditions.

(3) In some situations, an in vivo bioavailability study in humans may preferably and more properly be done in suitable patients. Critically ill patients shall not be included in an in vivo bioavailability study unless the attending physician determines that there is a potential benefit to the patient.

(b) *Basic design.* The basic design of an in vivo bioavailability study is determined by the following:

(1) The scientific questions to be answered.

(2) The nature of the reference material and the dosage form to be tested.

(3) The availability of analytical methods.

(4) Benefit-risk considerations in regard to testing in humans.

(c) *Comparison to a reference material.* In vivo bioavailability testing of a drug product shall be in comparison to an appropriate reference material unless some other approach is more appropriate for valid scientific reasons.

(d) *Previously unmarketed active drug ingredients or therapeutic moieties.* (1) The purpose of an in vivo bioavailability study involving a drug product containing an active drug ingredient or therapeutic moiety that has not been approved for marketing is to determine:

(i) The bioavailability of the formulation proposed for marketing; and

(ii) The essential pharmacokinetic characteristics of the active drug ingredient or therapeutic moiety, such as the rate of absorption, the extent of absorption, the half-life of the therapeutic moiety in vivo, and the rate of excretion and/or metabolism. Dose proportionality of the active drug ingredient or the therapeutic moiety needs to be established after single-dose administration and in certain instances after multiple-dose administration. This characterization is a necessary part of the investigation of the drug to support drug labeling.

(2) The reference material in such a bioavailability study should be a solution or suspension containing the same quantity of the active drug ingredient or therapeutic moiety as the formulation proposed for marketing.

(3) The reference material should be administered by the same route as the formulation proposed for marketing unless an alternative or additional route is necessary to answer the scientific question under study. For example, in the case of an active drug ingredient or therapeutic moiety that is poorly absorbed after oral administration, it may be necessary to compare

the oral dosage form proposed for marketing with the active drug ingredient or therapeutic moiety administered in solution both orally and intravenously.

(e) *New formulations of active drug ingredients or therapeutic moieties approved for marketing.* (1) The purpose of an in vivo bioavailability study involving a drug product that is a new formulation, a new dosage form, or a new salt or ester of an active drug ingredient or therapeutic moiety that has been approved for marketing is to:

(i) Determine the bioavailability of the new formulation, new dosage form, or new salt or ester relative to an appropriate reference material; and

(ii) Define the pharmacokinetic parameters of the new formulation, new dosage form, or new salt or ester to establish dosage recommendation.

(2) The selection of the reference material(s) in such a bioavailability study depends upon the scientific questions to be answered, the data needed to establish comparability to a currently marketed drug product, and the data needed to establish dosage recommendations.

(3) The reference material should be taken from a current batch of a drug product that is the subject of an approved new drug application and that contains the same active drug ingredient or therapeutic moiety, if the new formulation, new dosage form, or new salt or ester is intended to be comparable to or to meet any comparative labeling claims made in relation to the drug product that is the subject of an approved new drug application.

(f) *Controlled release formulations.* (1) The purpose of an in vivo bioavailability study involving a drug product for which a controlled release claim is made is to determine if all of the following conditions are met:

(i) The drug product meets the controlled release claims made for it.

(ii) The bioavailability profile established for the drug product rules out the occurrence of any dose dumping.

(iii) The drug product's steady-state performance is equivalent to a currently marketed noncontrolled release or controlled release drug product that contains the same active drug ingredient or therapeutic moiety and that is subject to an approved full new drug application.

(iv) The drug product's formulation provides consistent pharmacokinetic performance between individual dosage units.

(2) The reference material(s) for such a bioavailability study shall be chosen to permit an appropriate scientific evaluation of the controlled release claims made for the drug product. The reference material shall be one of the following or any combination thereof:

(i) A solution or suspension of the active drug ingredient or therapeutic moiety.

(ii) A currently marketed noncontrolled release drug product containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling of the noncontrolled release drug product.

(iii) A currently marketed controlled release drug product subject to an approved full new drug application containing the same active drug ingredient or therapeutic moiety and administered according to the dosage recommendations in the labeling proposed for the controlled release drug product.

(iv) A reference material other than one set forth in paragraph (f)(2) (i), (ii) or (iii) of this section that is appropriate for valid scientific reasons.

(g) *Combination drug products.* (1) Generally, the purpose of an in vivo bioavailability study involving a combination drug product is to determine if the rate and extent of absorption of each active drug ingredient or therapeutic moiety in the combination drug product is equivalent to the rate and extent of absorption of each active drug ingredient or therapeutic moiety administered concurrently in separate single-ingredient preparations.

(2) The reference material in such a bioavailability study should be two or more currently marketed, single-ingredient drug products each of which contains one of the active drug ingredients or therapeutic moieties in the combination drug product. The Food and Drug Administration may, for valid scientific reasons, specify that the reference material shall be a combination drug product that is the subject of an approved new drug application.

(3) The Food and Drug Administration may permit a bioavailability study involving a combination drug product to determine the rate and extent of absorption of selected, but not all, active drug ingredients or therapeutic moieties in the combination drug product. The Food and Drug Administration may permit this determination if the pharmacokinetics and the interactions of the active drug ingredients or therapeutic moieties in the combination drug product are well known and the therapeutic activity of the combination drug product is generally recognized to reside in only one of the active drug ingredients or therapeutic moieties, e.g., ampicillin in an ampicillin-probenecid combination drug product.

(h) *Use of a placebo as the reference material.* Where appropriate or where necessary to demonstrate the sensitivity of the test, the reference material in a bioavailability study may be a placebo if:

(1) The study measures the therapeutic or acute pharmacological effect of the active drug ingredient or therapeutic moiety; or

(2) The study is a clinical trial to establish the safety and effectiveness of the drug product.

(i) *Standards for test drug product and reference material.* (1) Both the drug product to be tested and the reference material, if it is another drug product, shall be shown to meet all compendial or other applicable standards of identity, strength, quality, and purity, including potency and, where applicable, content uniformity, disintegration times, and dissolution rates.

(2) Samples of the drug product to be tested shall be manufactured using the same equipment and under the same conditions as those used for full-scale production.

§ 320.26 Guidelines on the design of a single-dose in vivo bioavailability study.

(a) *Basic principles.* (1) An in vivo bioavailability study should be a single-dose comparison of the drug product to be tested and the appropriate reference material conducted in normal adults.

(2) The test product and the reference material should be administered to subjects in the fasting state, unless some other approach is more appropriate for valid scientific reasons.

(b) *Study design.* (1) A single-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period.

(2) Unless some other approach is appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured in the blood or urine; or

(ii) At least three times the half-life of decay of the acute pharmacological effect.

(c) *Collection of blood samples.* (1) When comparison of the test product and the reference material is to be based on blood concentration time curves, unless some other approach is more appropriate for valid scientific reasons, blood samples should be taken with sufficient frequency to permit an estimate of both:

(i) The peak concentration in the blood of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured; and

(ii) The total area under the curve for a time period at least three times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(2) In a study comparing oral dosage forms, the sampling times should be identical.

(3) In a study comparing an intravenous dosage form and an oral dosage form, the sampling times should be those needed to describe both:

(i) The distribution and elimination phase of the intravenous dosage form; and

(ii) The absorption and elimination phase of the oral dosage form.

(4) In a study comparing drug delivery systems other than oral or intravenous dosage forms with an appropriate reference standard, the sampling times should be based on valid scientific reasons.

(d) *Collection of urine samples.* When comparison of the test product and the

reference material is to be based on cumulative urinary excretion-time curves, unless some other approach is more appropriate for valid scientific reasons, samples of the urine should be collected with sufficient frequency to permit an estimate of the rate and extent of urinary excretion of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured.

(e) *Measurement of an acute pharmacological effect.* (1) When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to permit a reasonable estimate of the total area under the curve for a time period at least three times the half-life of decay of the pharmacological effect, unless some other approach is more appropriate for valid scientific reasons.

(2) The use of an acute pharmacological effect to determine bioavailability may further require demonstration of dose-related response. In such a case, bioavailability may be determined by comparison of the dose-response curves as well as the total area under the acute pharmacological effect-time curves for any given dose.

§ 320.27 Guidelines on the design of a multiple-dose in vivo bioavailability study.

(a) *Basic principles.* (1) In selected circumstances it may be necessary for the test product and the reference material to be compared after repeated administration to determine steady-state levels of the active drug ingredient or therapeutic moiety in the body.

(2) The test product and the reference material should be administered to subjects in the fasting or nonfasting state, depending upon the conditions reflected in the proposed labeling of the test product.

(3) A multiple-dose study may be required to determine the bioavailability of a drug product in the following circumstances:

(i) There is a difference in the rate of absorption but not in the extent of absorption.

(ii) There is excessive variability in bioavailability from subject to subject.

(iii) The concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in the blood resulting from a single dose is too low for accurate determination by the analytical method.

(iv) The drug product is a controlled release dosage form.

(b) *Study design.* (1) A multiple-dose study should be crossover in design, unless a parallel design or other design is more appropriate for valid scientific reasons, and should provide for a drug elimination period if steady-state conditions are not achieved.

(2) A multiple-dose study is not required to be of crossover design if the study is to establish dose proportionality under a multiple-dose regimen or to establish the pharmacokinetic profile of a new drug product, a new drug delivery system, or a controlled release dosage form.

(3) If a drug elimination period is required, unless some other approach is more appropriate for valid scientific reasons, the drug elimination period should be either:

(i) At least five times the half-life of the active drug ingredient or therapeutic moiety, or its metabolite(s), measured in the blood or urine; or

(ii) At least five times the half-life of decay of the acute pharmacological effect.

(c) *Achievement of steady-state conditions.* Whenever a multiple-dose study is conducted, unless some other approach is more appropriate for valid scientific reasons, sufficient doses of the test product and reference material should be administered in accordance with the labeling to achieve steady-state conditions.

(d) *Collection of blood or urine samples.* (1) Whenever comparison of the test product and the reference material is to be based on blood concentration-time curves at steady-state, sufficient samples of blood should be taken to define adequately the maximum (Cmax) and minimum (Cmin) blood concentrations on 2 or more consecutive days to establish that steady-state conditions are achieved.

(2) Whenever comparison of the test product and the reference material is to be based on cumulative urinary excretion-time curves at steady-state,

sufficient samples of urine should be taken to define the rate and extent of urinary excretion on 2 or more consecutive days to establish that steady-state conditions are achieved.

(3) A more complete characterization of the blood concentration or urinary excretion rate during the absorption and elimination phases of a single dose administered at steady-state is encouraged to permit estimation of the total area under concentration-time curves or cumulative urinary excretion-time curves and to obtain pharmacokinetic information, e.g., half-life or blood clearance, that is essential in preparing adequate labeling for the drug product.

(e) *Steady-state parameters.* (1) In certain instances, e.g., in a study involving a new drug entity, blood clearances at steady-state obtained in a multiple-dose study should be compared to blood clearances obtained in a single-dose study to support adequate dosage recommendations.

(2) In a linear system, the area under the blood concentration-time curve during a dosing interval in a multiple-dose steady-state study is directly proportional to the fraction of the dose absorbed and is equal to the corresponding ''zero to infinity'' area under the curve for a single-dose study. Therefore, when steady-state conditions are achieved, a comparison of blood concentrations during a dosing interval may be used to define the fraction of the active drug ingredient or therapeutic moiety absorbed.

(3) Other methods based on valid scientific reasons should be used to determine the bioavailability of a drug product having dose-dependent kinetics (non-linear system).

(f) *Measurement of an acute pharmacological effect.* When comparison of the test product and the reference material is to be based on acute pharmacological effect-time curves, measurements of this effect should be made with sufficient frequency to demonstrate a maximum effect and a lack of significant difference between the test product and the reference material.

§ 320.28 Correlation of bioavailability with an acute pharmacological effect or clinical evidence.

Correlation of in vivo bioavailability data with an acute pharmacological effect or clinical evidence of safety and effectiveness may be required if needed to establish the clinical significance of a special claim, e.g., in the case of a controlled release preparation.

§ 320.29 Analytical methods for an in vivo bioavailability study.

(a) The analytical method used in an in vivo bioavailability study to measure the concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in body fluids or excretory products, or the method used to measure an acute pharmacological effect shall be demonstrated to be accurate and of sufficient sensitivity to measure, with appropriate precision, the actual concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), achieved in the body.

(b) When the analytical method is not sensitive enough to measure accurately the concentration of the active drug ingredient or therapeutic moiety, or its metabolite(s), in body fluids or excretory products produced by a single dose of the test product, two or more single doses may be given together to produce higher concentration if the requirements of § 320.31 are met.

§ 320.30 Inquiries regarding bioavailability and bioequivalence requirements and review of protocols by the Food and Drug Administration.

(a) The Commissioner of Food and Drugs strongly recommends that, to avoid the conduct of an improper study and unnecessary human research, any person planning to conduct a bioavailability or bioequivalence study submit the proposed protocol for the study to FDA for review prior to the initiation of the study.

(b) FDA may review a proposed protocol for a bioavailability or bioequivalence study and will offer advice with respect to whether the following conditions are met:

(1) The design of the proposed bioavailability or bioequivalence study is appropriate.

(2) The reference material to be used in the bioavailability or bioequivalence study is appropriate.

(3) The proposed chemical and statistical analytical methods are adequate.

(c)(1) General inquiries relating to in vivo bioavailability requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Biopharmaceutics (HFD–420), 5600 Fishers Lane, Rockville, MD 20857.

(2) General inquiries relating to bioequivalence requirements and methodology shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Bioequivalence (HFD–650), 5600 Fishers Lane, Rockville, MD 20857.

[57 FR 18000, Apr. 28, 1992]

§ 320.31 Applicability of requirements regarding an "Investigational New Drug Application."

(a) Any person planning to conduct an in vivo bioavailability or bioequivalence study in humans shall submit an ''Investigational New Drug Application'' (IND) if:

(1) The test product contains a new chemical entity as defined in § 314.108(a) of this chapter; or

(2) The study involves a radioactively labeled drug product; or

(3) The study involves a cytotoxic drug product.

(b) Any person planning to conduct a bioavailability study in humans using a drug product that contains an already approved, non-new chemical entity shall submit an IND if the study is one of the following:

(1) A single-dose study in normal subjects or patients where either the maximum single or total daily dose exceeds that specified in the labeling of the drug product that is the subject of an approved new drug application or abbreviated new drug application.

(2) A multiple-dose study in normal subjects or patients where either the single or total daily dose exceeds that specified in the labeling of the drug product that is the subject of an approved new drug application or abbreviated new drug application.

(3) A multiple-dose study on a controlled release product on which no single-dose study has been completed.

(c) The provisions of parts 50, 56, and 312 of this chapter are applicable to any bioavailability or bioequivalence study in humans conducted under an IND.

(d) A bioavailability or bioequivalence study in humans other than one described in paragraphs (a) through (c) of this section is exempt from the requirements of part 312 of this chapter if the following conditions are satisfied:

(1) If the study is one described under § 320.38(b) or § 320.63, the person conducting the study, including any contract research organization, shall retain reserve samples of any test article and reference standard used in the study and release the reserve samples to FDA upon request, in accordance with, and for the period specified in, § 320.38; and

(2) An in vivo bioavailability or bioequivalence study in humans shall be conducted in compliance with the requirements for institutional review set forth in part 56 of this chapter, and informed consent set forth in part 50 of this chapter.

[57 FR 18000, Apr. 28, 1992, as amended at 58 FR 25927, Apr. 28, 1993]

§ 320.32 Procedures for establishing or amending a bioequivalence requirement.

(a) The Food and Drug Administration, on its own initiative or in response to a petition by an interested person, may propose and promulgate a regulation to establish a bioequivalence requirement for a product not subject to section 505(j) of the act if it finds there is well-documented evidence that specific pharmaceutical equivalents or pharmaceutical alternatives intended to be used interchangeably for the same therapeutic effect:

(1) Are not bioequivalent drug products; or

(2) May not be bioequivalent drug products based on the criteria set forth in § 320.33; or

(3) May not be bioequivalent drug products because they are members of a class of drug products that have close

structural similarity and similar physicochemical or pharmacokinetic properties to other drug products in the same class that FDA finds are not bioequivalent drug products.

(b) FDA shall include in a proposed rule to establish a bioequivalence requirement the evidence and criteria set forth in § 320.33 that are to be considered in determining whether to issue the proposal. If the rulemaking is proposed in response to a petition, FDA shall include in the proposal a summary and analysis of the relevant information that was submitted in the petition as well as other available information to support the establishment of a bioequivalence requirement.

(c) FDA, on its own initiative or in response to a petition by an interested person, may propose and promulgate an amendment to a bioequivalence requirement established under this subpart.

[57 FR 18000, Apr. 28, 1992]

§ 320.33 Criteria and evidence to assess actual or potential bioequivalence problems.

The Commissioner of Food and Drugs shall consider the following factors, when supported by well-documented evidence, to identify specific pharmaceutical equivalents and pharmaceutical alternatives that are not or may not be bioequivalent drug products.

(a) Evidence from well-controlled clinical trials or controlled observations in patients that such drug products do not give comparable therapeutic effects.

(b) Evidence from well-controlled bioequivalence studies that such products are not bioequivalent drug products.

(c) Evidence that the drug products exhibit a narrow therapeutic ratio, e.g., there is less than a 2-fold difference in median lethal dose (LD_{50}) and median effective dose (ED_{50}) values, or have less than a 2-fold difference in the minimum toxic concentrations and minimum effective concentrations in the blood, and safe and effective use of the drug products requires careful dosage titration and patient monitoring.

(d) Competent medical determination that a lack of bioequivalence would have a serious adverse effect in the treatment or prevention of a serious disease or condition.

(e) Physicochemical evidence that:

(1) The active drug ingredient has a low solubility in water, e.g., less than 5 milligrams per 1 milliliter, or, if dissolution in the stomach is critical to absorption, the volume of gastric fluids required to dissolve the recommended dose far exceeds the volume of fluids present in the stomach (taken to be 100 milliliters for adults and prorated for infants and children).

(2) The dissolution rate of one or more such products is slow, e.g., less than 50 percent in 30 minutes when tested using either a general method specified in an official compendium or a paddle method at 50 revolutions per minute in 900 milliliters of distilled or deionized water at 37° C, or differs significantly from that of an appropriate reference material such as an identical drug product that is the subject of an approved full new drug application.

(3) The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability.

(4) Certain physical structural characteristics of the active drug ingredient, e.g., polymorphic forms, conforms, solvates, complexes, and crystal modifications, dissolve poorly and this poor dissolution may affect absorption.

(5) Such drug products have a high ratio of excipients to active ingredients, e.g., greater than 5 to 1.

(6) Specific inactive ingredients, e.g., hydrophilic or hydrophobic excipients and lubricants, either may be required for absorption of the active drug ingredient or therapeutic moiety or, alternatively, if present, may interfere with such absorption.

(f) Pharmacokinetic evidence that:

(1) The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the gastrointestinal tract or is absorbed from a localized site.

(2) The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor, e.g., less than 50 percent, ordinarily in comparison to an intravenous dose, even when

it is administered in pure form, e.g., in solution.

(3) There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the process of absorption (first-class metabolism) so the therapeutic effect and/or toxicity of such drug product is determined by the rate as well as the degree of absorption.

(4) The therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness.

(5) The active drug ingredient or therapeutic moiety is unstable in specific portions of the gastrointestinal tract and requires special coatings or formulations, e.g., buffers, enteric coatings, and film coatings, to assure adequate absorption.

(6) The drug product is subject to dose dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to bioequivalence.

[42 FR 1635, Jan. 7, 1977. Redesignated and amended at 57 FR 18001, Apr. 28, 1992]

§ 320.34 Requirements for batch testing and certification by the Food and Drug Administration.

(a) If the Commissioner determines that individual batch testing by the Food and Drug Administration is necessary to assure that all batches of the same drug product meet an appropriate in vitro test, he shall include in the bioequivalence requirement a requirement for manufacturers to submit samples of each batch to the Food and Drug Administration and to withhold distribution of the batch until notified by the Food and Drug Administration that the batch may be introduced into interstate commerce.

(b) The Commissioner will ordinarily terminate a requirement for a manufacturer to submit samples for batch testing on a finding that the manufacturer has produced four consecutive batches that were tested by the Food and Drug Administration and found to meet the bioequivalence requirement, unless the public health requires that batch testing be extended to additional batches.

[42 FR 1635, Jan. 7, 1977. Redesignated at 57 FR 18001, Apr. 28, 1992]

§ 320.35 Requirements for in vitro testing of each batch.

If a bioequivalence requirement specifies a currently available in vitro test or an in vitro bioequivalence standard comparing the drug product to a reference standard, the manufacturer shall conduct the test on a sample of each batch of the drug product to assure batch-to-batch uniformity.

[42 FR 1635, Jan. 7, 1977. Redesignated at 57 FR 18001, Apr. 28, 1992]

§ 320.36 Requirements for maintenance of records of bioequivalence testing.

(a) All records of in vivo or in vitro tests conducted on any marketed batch of a drug product to assure that the product meets a bioequivalence requirement shall be maintained by the manufacturer for at least 2 years after the expiration date of the batch and submitted to the Food and Drug Administration on request.

(b) Any person who contracts with another party to conduct a bioequivalence study from which the data are intended to be submitted to FDA as part of an application submitted under part 314 of this chapter shall obtain from the person conducting the study sufficient accurate financial information to allow the submission of complete and accurate financial certifications or disclosure statements required under part 54 of this chapter and shall maintain that information and all records relating to the compensation given for that study and all other financial interest information required under part 54 of this chapter for 2 years after the date of approval of the application. The person maintaining these records shall, upon request for any properly authorized officer or employee of the Food and Drug Administration, at reasonable time, permit such officer or employee to have access to and copy and verify these records.

[42 FR 1635, Jan. 7, 1977. Redesignated at 57 FR 18001, Apr. 28, 1992, as amended at 63 FR 5252, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5252, Feb. 2, 1998, § 320.36 was amended by designating the existing text as paragraph (a) and by adding new paragraph (b), effective Feb. 2, 1999.

§ 320.38 Retention of bioavailability samples.

(a) The applicant of an application or supplemental application submitted under section 505 or 507 of the Federal Food, Drug, and Cosmetic Act, or, if bioavailability testing was performed under contract, the contract research organization shall retain an appropriately identified reserve sample of the drug product for which the applicant is seeking approval (test article) and of the reference standard used to perform an in vivo bioavailability study in accordance with and for the studies described in paragraph (b) of this section that is representative of each sample of the test article and reference standard provided by the applicant for the testing.

(b) Reserve samples shall be retained for the following test articles and reference standards and for the studies described:

(1) If the formulation of the test article is the same as the formulation(s) used in the clinical studies demonstrating substantial evidence of safety and effectiveness for the test article's claimed indications, a reserve sample of the test article used to conduct an in vivo bioavailability study comparing the test article to a reference oral solution, suspension, or injection.

(2) If the formulation of the test article differs from the formulation(s) used in the clinical studies demonstrating substantial evidence of safety and effectiveness for the test article's claimed indications, a reserve sample of the test article and of the reference standard used to conduct an in vivo bioequivalence study comparing the test article to the formulation(s) (reference standard) used in the clinical studies.

(3) For a new formulation, new dosage form, or a new salt or ester of an active drug ingredient or therapeutic moiety that has been approved for marketing, a reserve sample of the test article and of the reference standard used to conduct an in vivo bioequivalence study comparing the test article to a marketed product (reference standard) that contains the same active drug ingredient or therapeutic moiety.

(c) Each reserve sample shall consist of a sufficient quantity to permit FDA to perform five times all of the release tests required in the application or supplemental application.

(d) Each reserve sample shall be adequately identified so that the reserve sample can be positively identified as having come from the same sample as used in the specific bioavailability study.

(e) Each reserve sample shall be stored under conditions consistent with product labeling and in an area segregated from the area where testing is conducted and with access limited to authorized personnel. Each reserve sample shall be retained for a period of at least 5 years following the date on which the application or supplemental application is approved, or, if such application or supplemental application is not approved, at least 5 years following the date of completion of the bioavailability study in which the sample from which the reserve sample was obtained was used.

(f) Authorized FDA personnel will ordinarily collect reserve samples directly from the applicant or contract research organization at the storage site during a preapproval inspection. If authorized FDA personnel are unable to collect samples, FDA may require the applicant or contract research organization to submit the reserve samples to the place identified in the agency's request. If FDA has not collected or requested delivery of a reserve sample, or if FDA has not collected or requested delivery of any portion of a reserve sample, the applicant or contract research organization shall retain the sample or remaining sample for the 5-year period specified in paragraph (e) of this section.

(g) Upon release of the reserve samples to FDA, the applicant or contract research organization shall provide a written assurance that, to the best knowledge and belief of the individual executing the assurance, the reserve samples came from the same samples as used in the specific bioavailability or bioequivalence study identified by the agency. The assurance shall be executed by an individual authorized to act for the applicant or contract research organization in releasing the reserve samples to FDA.

(h) A contract research organization may contract with an appropriate, independent third party to provide storage of reserve samples provided that the sponsor of the study has been notified in writing of the name and address of the facility at which the reserve samples will be stored.

(i) If a contract research organization conducting a bioavailability or bioequivalence study that requires reserve sample retention under this section or § 320.63 goes out of business, it shall transfer its reserve samples to an appropriate, independent third party, and shall notify in writing the sponsor of the study of the transfer and provide the study sponsor with the name and address of the facility to which the reserve samples have been transferred.

[58 FR 25927, Apr. 28, 1993]

§ 320.63 Retention of bioequivalence samples.

The applicant of an abbreviated application or a supplemental application submitted under section 505 or 507 of the Federal Food, Drug, and Cosmetic Act, or, if bioequivalence testing was performed under contract, the contract research organization shall retain reserve samples of any test article and reference standard used in conducting an in vivo or in vitro bioequivalence study required for approval of the abbreviated application or supplemental application. The applicant or contract research organization shall retain the reserve samples in accordance with, and for the period specified in, § 320.38 and shall release the reserve samples to FDA upon request in accordance with § 320.38.

[58 FR 25928, Apr. 28, 1993]

PART 328—OVER-THE-COUNTER DRUG PRODUCTS INTENDED FOR ORAL INGESTION THAT CONTAIN ALCOHOL

Subpart A—General Provisions

Sec.
328.1 Scope.
328.3 Definitions.

Subpart B—Ingredients

328.10 Alcohol.

Subpart C—Labeling

328.50 Principal display panel of all OTC drug products intended for oral ingestion that contain alcohol.

AUTHORITY: Secs. 201, 301, 501, 502, 503, 505, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 331, 351, 352, 353, 355, 371).

SOURCE: 60 FR 13595, Mar. 13, 1995, unless otherwise noted.

Subpart A—General Provisions

§ 328.1 Scope.

Reference in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 328.3 Definitions.

As used in this part:

(a) *Alcohol* means the substance known as ethanol, ethyl alcohol, or Alcohol, USP.

(b) *Inactive ingredient* means any component of a product other than an active ingredient as defined in § 210.3(b)(7) of this chapter.

Subpart B—Ingredients

§ 328.10 Alcohol.

(a) Any over-the-counter (OTC) drug product intended for oral ingestion shall not contain alcohol as an inactive ingredient in concentrations that exceed those established in this part, unless a specific exemption, as provided in paragraph (e) or (f) of this section, has been approved.

(b) For any OTC drug product intended for oral ingestion and labeled for use by adults and children 12 years of age and over, the amount of alcohol in the product shall not exceed 10 percent.

(c) For any OTC drug product intended for oral ingestion and labeled for use by children 6 to under 12 years of age, the amount of alcohol in the product shall not exceed 5 percent.

(d) For any OTC drug product intended for oral ingestion and labeled for use by children under 6 years of age, the amount of alcohol in the product shall not exceed 0.5 percent.

(e) The Food and Drug Administration will grant an exemption from

paragraphs (b), (c), and (d) of this section where appropriate, upon petition under the provisions of § 10.30 of this chapter. Appropriate cause, such as a specific solubility or manufacturing problem, must be adequately documented in the petition. Decisions with respect to requests for exemption shall be maintained in a permanent file for public review by the Dockets Management Branch (HFA–305), Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857.

(f) Ipecac syrup is exempt from the provisions of paragraph (d) of this section.

(g) The following drugs are temporarily exempt from the provisions of paragraphs (b), (c), and (d) of this section:

(1) Aromatic Cascara Fluidextract.

(2) Cascara Sagrada Fluidextract.

(3) Orally ingested homeopathic drug products.

[60 FR 13595, Mar. 13, 1995, as amended at 61 FR 58630, Nov. 18, 1996]

Subpart C—Labeling

§ 328.50 Principal display panel of all OTC drug products intended for oral ingestion that contain alcohol.

(a) The amount (percentage) of alcohol present in a product shall be stated in terms of percent volume of absolute alcohol at 60 ° F (15.56 ° C) in accordance with § 201.10(d)(2) of this chapter.

(b) A statement expressing the amount (percentage) of alcohol present in a product shall appear prominently and conspicuously on the ''principal display panel,'' as defined in § 201.60 of this chapter. For products whose principal display panel is on the immediate container label and that are not marketed in another retail package (e.g., an outer box), the statement of the percentage of alcohol present in the product shall appear prominently and conspicuously on the ''principal display panel'' of the immediate container label.

(c) For products whose principal display panel is on the retail package and the retail package is not the immediate container, the statement of the percentage of alcohol present in the product shall also appear on the immediate container label; it may appear anywhere on that label in accord with section 502(e) of the Federal Food, Drug, and Cosmetic Act.

(d) The statement expressing the amount (percentage) of alcohol present in the product shall be in a size reasonably related to the most prominent printed matter on the panel or label on which it appears, and shall be in lines generally parallel to the base on which the package rests as it is designed to be displayed.

(e) For a product to state in its labeling that it is ''alcohol free,'' it must contain no alcohol (0 percent).

(f) For any OTC drug product intended for oral ingestion containing over 5 percent alcohol and labeled for use by adults and children 12 years of age and over, the labeling shall contain the following statement in the directions section: ''Consult a physician for use in children under 12 years of age.''

(g) For any OTC drug product intended for oral ingestion containing over 0.5 percent alcohol and labeled for use by children ages 6 to under 12 years of age, the labeling shall contain the following statement in the directions section: ''Consult a physician for use in children under 6 years of age.''

(h) When the direction regarding age in paragraph (e) or (f) of this section differs from an age-limiting direction contained in any OTC drug monograph in this chapter, the direction containing the more stringent age limitation shall be used.

PART 329—HABIT-FORMING DRUGS

Subpart A—Derivatives Designated as Habit Forming

Sec.

329.1 Habit-forming drugs which are chemical derivatives of substances specified in section 502(d) of the Federal Food, Drug, and Cosmetic Act.

Subpart B—Labeling

329.10 Labeling requirements for habit-forming drugs.

Subpart C—Exemptions

329.20 Exemption of certain habit-forming drugs from prescription requirements.

AUTHORITY: 21 U.S.C. 352, 353, 355, 371.

SOURCE: 39 FR 11736, Mar. 29, 1974, unless otherwise noted.

Subpart A—Derivatives Designated as Habit Forming

§ 329.1 Habit-forming drugs which are chemical derivatives of substances specified in section 502(d) of the Federal Food, Drug, and Cosmetic Act.

Each of the following chemical derivatives of a substance named in section 502(d) of the Federal Food, Drug, and Cosmetic Act is hereby designated as habit forming:

Chemical description of derivative	Common or official name of chemical derivative or its salts	Some trade or other names of chemical derivative or its salts [1]
PARENT SUBSTANCE—BARBITURIC ACID		
5-Allyl-5-*sec*-butylbarbituric acid [2]	Talbutal	Lotusate.
5-Allyl-5-cyclopentenylbarbituric acid		Cyclopal.
		Cyclopen.
5-Allyl-5-isobutylbarbituric acid	Allylbarbituric acid	Sandoptal.
	Allylisobutylbarbituric acid..	
5-Allyl-5-isopropylbarbituric acid	Aprobarbital	Alurate.
	Allylisopropylbarbituric acid	Numal.
	Allylisopropylmalonylurea..	
5-Allyl-5-isopropyl-1-methylbarbituric acid		Narconumal.
5-Allyl-5-(1-methylbutyl)barbituric acid	Secobarbital sodium	Seconal Sodium.
	Soluble secobarbital	Evronal Sodium.
5-Allyl-5-(1-methylbutyl)-2-thiobarbituric acid	Sodium thiamylal	Surital Sodium.
5-Allyl-1-methyl-5-(1-methyl-2-pentynyl) barbituric acid.	Sodium methohexital	Brevital Sodium.
5-(2-Bromoallyl)-5-isoprophyl-1-methylbarbituric acid.		Eunarcon.
5-(2-Bromoallyl)-5-(1-methylbutyl)-barbituric acid	β-Bromoallyl *sec*-amylbarbituric acid	Sigmodal.
		Rectidon.
		R239.
5-*sec*-Butyl-5-(2-bromoallyl)-barbituric acid	Butallylonal	Pernoston.
		Pernocton.
5-(1-Cyclohepten-1-yl)-5-ethylbarbituric acid	Heptabarbital	Medomin.
5,5-Diallylbarbituric acid	Diallyl barbituric acid	Dial.
		Allobarbital.
		Allobarbitone.
		Curral.
		Diadol.
5,5-Diethylbarbituric acid	Barbital	Deba.
	Barbitone	Dormonal.
	Diethylbarbituric acid	Hypnogene.
	Diethylmalonylurea	Malonal.
		Medinal.
		Sedeval.
		Veronal.
		Uronal.
		Vesperal.
5,5-Diethyl-1-methylbarbituric acid	Metharbital	Gemonil.
1,5-Dimethyl-5-(1-cyclohexenyl)-barbituric acid	Hexobarbital sodium	Cyclonal Sodium.
		Dorico Soluble.
		Evipal Sodium.
		Evipan Sodium.
		Hexanastab.
		Hexobarbitone Sodium.
		Methenexyl Sodium.
5,5-Dipropylbarbituric acid	Dipropylbarbituric acid	Proponal.
5-Ethyl-5-butylbarbituric acid	Butethal	Etoval.
	Butobarbital	Neonal Butobarbital.
		Soneryl.
5-Ethyl-5-*sec*-butylbarbituric acid	Butabarbital sodium	Butisol Sodium.

Chemical description of derivative	Common or official name of chemical derivative or its salts	Some trade or other names of chemical derivative or its salts [1]
5-Ethyl-5-(1-cyclohexenyl)-barbituric acid	Cyclobarbital	Cyclobarbitone. Namuron. Palinum. Phanodorm. Phanodorn. Tetrahydro phenobarbital.
5-Ethyl-5-cyclopentenyl-barbituric acid		Pentenal.
5-Ethyl-5-hexylbarbituric acid	Hexethal sodium	Hebaral. Ortal Sodium.
5-Ethyl-5-isoamylbarbituric acid	Amobarbital	Amytal.
5-Ethyl-5-isopropylbarbituric acid	Probarbital	Ipral.
5-Ethyl-5-(1-methylbutyl)-barbituric acid	Pentobarbital sodium	844.
	Soluble pentobarbital	Embutal. Nembutal. Napethal. Pentyl.
5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric acid	Thiopental sodium	Intraval Sodium.
	Thiopentone sodium	Nesdonal Sodium. Pentothal Sodium. Thiothal Sodium.
5-Ethyl-5-(1-methyl-1-butenyl)-barbituric acid	Vinbarbital	Delvinal Sodium.
5-Ethyl-5-phenylbarbituric acid	Phenobarbital	Barbenyl.
	Phenobarbitone	Barbiphenyl.
	Phenylethylmalonylurea	Dormiral. Euneryl. Gardenal. Luminal. Nunol. Neurobarb. Phenonyl. Somonal.
5-Ethyl-5-phenyl-1-methylbarbituric acid	Mephobarbital	Mebaral. Phemitone. Prominal.
5-Ethyl-5-(1 piperidyl)-barbituric acid		Eldoral.
5-Isopropyl-5-(2-bromoallyl)-barbituric acid	Propallylonal	Noctal. Nostal.
5-(1-Methylbutyl)-5-[2-(methylthio)ethyl]-2-thiobarbituric acid.	Methitural (sodium salt)	Methioturiate. Neraval. Thiogenal.
5-Methyl-5-phenylbarbituric acid	Phenylmethylbarbituric acid	Rutonal.
All lithium, sodium, potassium, magnesium, calcium, strontium, and ammonium salts of the foregoing chemical derivatives of barbituric acid.		
PARENT SUBSTANCE—CANNABIS (MARIHUANA)		
	Extract of cannabis. Fluid extract of cannabis. Tincture of cannabis.	
PARENT SUBSTANCE—BROMAL		
Tribromoacetaldehyde hydrate	Bromal hydrate.	
Tribromomethane	Bromoform.	
2-(Tribromomethyl)-2-propanol	Tribromo-*tert*-butyl alcohol	Acetone-Bromoform. Brometone.
PARENT SUBSTANCE—CARBROMAL		
a-Bromo-*a*-ethylbutyryl-acetylurea	Acetylcarbromal	A basin. Acetyl Adalin. *N*-Acetyl-*N*-bromodiethylacetylurea. *N*-Acetyl-*N'*-*a*-bromo-*a*-ethylbutyryl carbamide.

Chemical description of derivative	Common or official name of chemical derivative or its salts	Some trade or other names of chemical derivative or its salts [1]
a-Bromoisovalerylurea	Bromisovalum	Bromisoval. *a*-Bromo-β-dimethyl-propanoylurea. Bromural. Bromvaletone. Brovalurea. B. V. U. Dormigene. Isobromyl. 2-Monobromoisovalerylurea. Pivadorm. Uvaleral.
a-Bromo-*a,a*-diethylacetamide	Diethylbromo acetamide	Neuronal.
a-Allylisovaleryl-urea		Allyl-isopropyl-acetyl-carbamide. (2-Isopropyl-4-pentenoyl)-urea. Sedormid.
PARENT SUBSTANCE—CHLORAL		
Trichloroacetaldehyde hydrate	Chloral	2,2,2-Trichloro-1,1-ethanediol.
	Chloral hydrate	Trichloroethylidene glycol.
Trichloroethylideneimine	Chloralimide.	
N-(β-Trichloro-*a*-hydroxyethyl)-formamide	Chloralformamide	Chloralamide. Chloramide.
a-(β-trichloro-*a*-hydroxyethyl)-*D*-glucoside	*a*-Chloralose	A-D-Glucochloralose. Anhydro-Glucochloral. Glucochloral. Chloralosone.
2-(Trichloromethyl)-2-propanol	Chlorbutanol	Acetone chloroform.
	Chlorbutol	Chloretone.
	Chlorobutanol	Methaform. Sedaform. 1,1,1-trichloro-2-methyl 2-propanol. β,β,β-trichloro-*tert*-butylalcohol.
PARENT SUBSTANCE—COCAINE		
All salts of cocaine obtained by combining cocaine with any acid.	Cocaine hydrochloride Cocainium chloride..	
PARENT SUBSTANCE—CODEINE		
Codeine methylbromide	Eucodin.	
Dihydrocodeinone		Dicodid.
Dihydrohydroxycodeinone	Eucodal	Oxycodone hydrochloride. 14-hydroxydihydrocodeinone.
All salts of the foregoing chemical derivatives of codeine obtained by combining any such derivative of codeine with any acid.		
PARENT SUBSTANCE—HEROIN		
All salts of heroin obtained by combining heroin with any acid.		
PARENT SUBSTANCE—MORPHINE		
Dihydromorphine	Paramorphan.	
Dihydromorphinone	Dihydromorphinone hydrochloride	Dilaudid. Dimorphone.
	Dihydromorphinonium chloride	Hydromorphone hydrochloride.
Ethylmorphine	Ethylmorphine hydrochloride Ethylmorphinium chloride..	Dionin.
All salts of the foregoing chemical derivatives of morphine and all salts of morphine obtained by combining any such derivative or morphine with any acid.		
PARENT SUBSTANCE—OPIUM		
	Extract of opium.	

Chemical description of derivative	Common or official name of chemical derivative or its salts	Some trade or other names of chemical derivative or its salts [1]
	Fluidextract of opium. Camphorated opium tincture. Deodorized opium tincture. Laudanum. Opium tincture. Paregoric. Tincture of opium.	
PARENT SUBSTANCE—PARALDEHYDE		
Metaldehyde.		
PARENT SUBSTANCE—SULFONMETHANE		
2,2-Diethylsulfonylbutane	Sulfonethylmethane	Diethylsulfonmethylethyl-methane. Ethylsulfonal. 2,2-*bis*-(Ethylsulfonyl)-butane. Methylsufonal. Sulfonethlylmethanum. Trional.
3,3-Diethylsulfonylpentane	Sulfondiethylmethane.	

[1] This list of trade or other names is not a complete list of the many proprietary names under which the designated habit-forming chemical derivatives are distributed.
[2] The name ''butalbital'' is obsolete for this compound; ''butalbital'' is the nonproprietary name assigned by the United States Adopted Name Council and the World Health Organization for 5-allyl-5-isobutylbarbituric acid.

Subpart B—Labeling

§ 329.10 Labeling requirements for habit-forming drugs.

(a)(1) The name of a substance or derivative required to be borne on the label of a drug by section 502(d) of the act shall be the common or usual name of such substance or derivative, unless it is designated solely by a name recognized in an official compendium and such designation complies with the provisions of section 502(c).

(2) A statement on the label of a drug of the name of a constituent, which constituent is a chemical derivative of a substance named in section 502(d) of the act, shall show the substance from which such constituent is derived and that such constituent is a derivative thereof.

(b) If the drug is in tablet, capsule, ampul, or other unit form, the statement of the quantity or proportion of such substance or derivative contained therein shall express the weight or measure of such substance or derivative in each such unit. If the drug is not in such unit form the statement shall express the weight or measure of such substance or derivative in a specified unit of weight or measure of the drug. Such statement shall be in terms which are informative to the ordinary consumer and user of the drug.

(c) The names and quantities or proportions of all such substances and derivatives, and the statement ''Warning—May be habit forming'', shall immediately follow (without intervening written, printed, or graphic matter) the name by which such drug is titled in the part or panel of the label thereof which is presented or displayed under customary conditions of purchase.

(d) A drug shall not be considered to be misbranded by reason of failure of its label to bear the statement ''Warning—May be habit forming'':

(1) If such drug is not suitable for internal use, and is distributed and sold exclusively for such external use as involves no possibility of habit formation; or

(2) If the only substance or derivative subject to section 502(d) of the act contained in such drug is chlorobutanol, which is present solely as a preservative and in a quantity not more than 0.5 percent by weight, and such drug is for parenteral use only; or

(3) If the only substance or derivative subject to section 502(d) of the act contained in such drug is chlorobutanol which is present as an analgesic or as an analgesic and a preservative in a quantity not more than 3.0 percent,

and such drug contains one or more other active ingredients and is for parenteral use only.

CROSS REFERENCE: For the Spanish-language version of the required labeling statement, see § 201.16(b) of this chapter.

[39 FR 11736, Mar. 29, 1974, as amended at 40 FR 13496, Mar. 27, 1975]

Subpart C—Exemptions

§ 329.20 Exemption of certain habit-forming drugs from prescription requirements.

The prescription-dispensing requirements of section 503(b)(1)(A) of the act are not necessary for the protection of the public health with respect to the following drugs subject to section 502(d):

(a) The following exempt narcotic preparations:

(1) Pharmaceutical preparations containing not more than 100 milligrams of opium per 100 milliliters or per 100 grams.

(2) Pharmaceutical preparations containing not more than 16.2 milligrams (¼ grain) morphine, or any of its salts, per 29.5729 cubic centimeters (1 fluid ounce) or per 28.3 grams (1 avoirdupois ounce);

(3) Pharmaceutical preparations containing not more than 64.8 milligrams (1 grain) codeine, or any of its salts, per 29.5729 cubic centimeters (1 fluid ounce) or per 28.3 grams (1 avoirdupois ounce);

(4) Pharmaceutical preparations containing not more than 32.4 milligrams (½ grain) dihydrocodeine, or any of its salts, per 29.5729 cubic centimeters (1 fluid ounce) or per 28.3 grams (1 avoirdupois ounce);

(5) Pharmaceutical preparations containing not more than 16.2 milligrams (¼ grain) ethylmorphine, or any of its salts, per 29.5729 cubic centimeters (1 fluid ounce) or per 28.3 grams (1 avoirdupois ounce);

Provided, That the preparations described in this paragraph contain one or more nonnarcotic active medicinal ingredients in sufficient proportion to confer upon the preparation valuable medicinal qualities other than those possessed by the narcotic drug alone.

(b) Drugs containing chlorobutanol, intended for external use only.

(c) Epinephrine solution, 1 percent, preserved with chlorobutanol and intended for use solely as a spray.

(d) Combination drugs listed in part 329 as exempted from section 511 of the act.

[39 FR 11736, Mar. 29, 1974, as amended at 55 FR 11581, Mar. 29, 1990]

PART 330—OVER-THE-COUNTER (OTC) HUMAN DRUGS WHICH ARE GENERALLY RECOGNIZED AS SAFE AND EFFECTIVE AND NOT MISBRANDED

Subpart A—General Provisions

Sec.
330.1 General conditions for general recognition as safe, effective and not misbranded.
330.2 Pregnancy–nursing warning.
330.3 Imprinting of solid oral dosage form drug products.
330.5 Drug categories.

Subpart B—Administrative Procedures

330.10 Procedures for classifying OTC drugs as generally recognized as safe and effective and not misbranded, and for establishing monographs.
330.11 NDA deviations from applicable monograph.
330.12 Status of over-the-counter (OTC) drugs previously reviewed under the Drug Efficacy Study (DESI).
330.13 Conditions for marketing ingredients recommended for over-the-counter (OTC) use under the OTC drug review.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 39 FR 11741, Mar. 29, 1974, unless otherwise noted.

Subpart A—General Provisions

§ 330.1 General conditions for general recognition as safe, effective and not misbranded.

An over-the-counter (OTC) drug listed in this subchapter is generally recognized as safe and effective and is not misbranded if it meets each of the conditions contained in this part and each of the conditions contained in any applicable monograph. Any product which fails to conform to each of the conditions contained in this part and

in an applicable monograph is liable to regulatory action.

(a) The product is manufactured in compliance with current good manufacturing practices, as established by parts 210 and 211 of this chapter.

(b) The establishment(s) in which the drug product is manufactured is registered, and the drug product is listed, in compliance with part 207 of this chapter. It is requested but not required that the number assigned to the product pursuant to part 207 of this chapter appear on all drug labels and in all drug labeling. If this number is used, it shall be placed in the manner set forth in part 207 of this chapter.

(c)(1) The product is labeled in compliance with chapter V of the act and subchapter C *et seq.* of this chapter. For purposes of § 201.61(b) of this chapter, the statement of identity of the product shall be the term or phrase used in the applicable monograph established in this part.

(2)(i) The label and labeling of the product contain in a prominent and conspicuous location the labeling describing the ''Indications'' that have been established in an applicable final monograph. At the option of the manufacturer, this labeling may be designated ''APPROVED USES,'' or be given a similar designation as permitted by this paragraph, each time it appears in the labeling, e.g., on the outer carton, inner bottle label, and on any package insert or display material. If the ''APPROVED USES'' or a similar designation is used, the labeling involved shall appear within a boxed area. Other applicable labeling established under this subchapter and subchapter C of this chapter may be included in the boxed area. If such other labeling is included, the boxed area shall be designated ''APPROVED INFORMATION'' rather than ''APPROVED USES.'' The ''indications'' information appearing in the boxed area shall be stated in the exact language of the monograph. Other information within the boxed area also shall be stated in exact language where exact language has been established and identified by quotation marks in an applicable monograph or by regulation (e.g., § 201.63 of this chapter). A statement that the information in the box was ''published by the Food and Drug Administration'' shall appear within the boxed area, or reasonably close by. In lieu of such statement, the designation of the boxed area may be modified to read: ''FDA APPROVED USES'' or ''FDA APPROVED INFORMATION,'' as appropriate, or ''USES (or ''INFORMATION'') APPROVED BY THE FOOD AND DRUG ADMINISTRATION,'' or other similar wording.

(ii) At the option of the manufacturer, as an alternative to the requirements of paragraph (c)(2)(i) of this section, the label and labeling of the product may contain in a prominent and conspicuous location other truthful and nonmisleading statements describing only those indications for use that have been established in an applicable monograph, subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act. Such labeling shall not be boxed and shall not contain the statements provided in paragraph (c)(2)(i) of this section relating to ''APPROVED USES,'' or ''APPROVED INFORMATION,'' or contain a statement that the labeling has been published by the Food and Drug Administration.

(iii) At the option of the manufacturer, the label and labeling may meet the boxed-area requirements of paragraph (c)(2)(i) of this section and, in addition, other truthful and nonmisleading statements describing only those indications for use that have been established in an applicable monograph may appear elsewhere in the labeling, that is, outside the boxed area, subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(iv) At the option of the manufacturer, more than one of the alternatives described in paragraphs (c)(2)(i), (ii), and (iii) may be used in separate labeling, e.g., container label, outer carton, package insert, display material, for a particular OTC drug

product provided each labeling complies with all applicable statutory and regulatory labeling requirements in all respects.

(v) The term ''prominent and conspicuous location'' as used in paragraphs (c)(2) (i) and (ii) of this section means that the labeling within the boxed or nonboxed area shall be presented and displayed in such a manner as to render it likely to be read as understood by the ordinary individual under customary conditions at both time of purchase and use.

(vi) Regardless of the alternative selected by the manufacturer to describe indications, paragraphs (c)(2)(i), (ii), and (iii) of this section require other labeling established under this subchapter and subchapter C of this chapter to be stated in the exact language where exact language has been established and identified by quotation marks in an applicable monograph or by regulation (e.g., § 201.63 of this chapter).

(d) The advertising for the product prescribes, recommends, or suggests its use only under the conditions stated in the labeling.

(e) The product contains only suitable inactive ingredients which are safe in the amounts administered and do not interfere with the effectiveness of the preparation or with suitable tests or assays to determine if the product meets its professed standards of identity, strength, quality, and purity. Color additives may be used only in accordance with section 721 of the act and subchapter A of this chapter.

(f) The product container and container components meet the requirements of § 211.94 of this chapter.

(g) The labeling for all drugs contains the general warning: ''Keep this and all drugs out of the reach of children.'' The labeling of drugs used for oral administation shall also state: ''In case of accidental overdose, seek professional assistance or contact a poison control center immediately.'' The labeling for drugs administered rectally or used topically shall state: ''In case of accidental ingestion, seek professional assistance or contact a Poison Control Center immediately.'' The Food and Drug Administration will grant an exemption from these general warnings where appropriate upon petition, which shall be maintained in a permanent file for public review by the Dockets Management Branch, Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857.

(h) Where no maximum daily dosage limit for an active ingredient is established in this part, it is used in a product at a level that does not exceed the amount reasonably required to achieve its intended effect.

(i) The following terms may be used interchangeably in any of the labeling established in parts 331 through 358 of this chapter:

(1) ''Ask'' or ''consult''.
(2) ''Assistance'' or ''help''.
(3) ''Clean'' or ''cleanse''.
(4) ''Continue'' or ''persist''.
(5) ''Continues'' or ''persists''.
(6) ''Doctor'' or ''physician''.
(7) ''Indication'' or ''use''.
(8) ''Indications'' or ''uses''.
(9) ''Lung'' or ''pulmonary''.

(j) It is recommended that the labeling of the product contain the quantitative amount of each active ingredient, expressed in terms of the dosage unit stated in the directions for use (e.g., tablet, teaspoonful).

[39 FR 11741, Mar. 29, 1974, as amended at 40 FR 11718, Mar. 13, 1975; 40 FR 13496, Mar. 27, 1975; 42 FR 15674, Mar. 22, 1977; 46 FR 8459, Jan. 27, 1981; 50 FR 8996, Mar. 6, 1985; 51 FR 16266, May 1, 1986; 55 FR 11581, Mar. 29, 1990; 59 FR 4000, Jan. 28, 1994; 59 FR 14365, Mar. 28, 1994]

§ 330.2 Pregnancy-nursing warning.

A pregnancy-nursing warning for OTC drugs is set forth under § 201.63 of this chapter.

[47 FR 54758, Dec. 3, 1982]

§ 330.3 Imprinting of solid oral dosage form drug products.

A requirement to imprint an identification code on solid oral dosage form drug products is set forth under part 206 of this chapter.

[58 FR 47959, Sept. 13, 1993]

§ 330.5 Drug categories.

Monographs promulgated pursuant to the provisions of this part shall be established in this part 330 and following

parts and shall cover the following designated categories:

(a) Antacids.
(b) Laxatives.
(c) Antidiarrheal products.
(d) Emetics.
(e) Antiemetics.
(f) Antiperspirants.
(g) Sunburn prevention and treatment products.
(h) Vitamin-mineral products.
(i) Antimicrobial products.
(j) Dandruff products.
(k) Oral hygiene aids.
(l) Hemorrhoidal products.
(m) Hematinics.
(n) Bronchodilator and antiasthmatic products.
(o) Analgesics.
(p) Sedatives and sleep aids.
(q) Stimulants.
(r) Antitussives.
(s) Allergy treatment products.
(t) Cold remedies.
(u) Antirheumatic products.
(v) Ophthalmic products.
(w) Contraceptive products.
(x) Miscellaneous dermatologic products.
(y) Dentifrices and dental products such as analgesics, antiseptics, etc.
(z) Miscellaneous (all other OTC drugs not falling within one of the above therapeutic categories).

Subpart B—Administrative Procedures

§ 330.10 Procedures for classifying OTC drugs as generally recognized as safe and effective and not misbranded, and for establishing monographs.

For purposes of classifying over-the-counter (OTC) drugs as drugs generally recognized among qualified experts as safe and effective for use and as not misbranded drugs, the following regulations shall apply:

(a) *Procedure for establishing OTC drug monographs*—(1) *Advisory review panels.* The Commissioner shall appoint advisory review panels of qualified experts to evaluate the safety and effectiveness of OTC drugs, to review OTC drug labeling, and to advise him on the promulgation of monographs establishing conditions under which OTC drugs are generally recognized as safe and effective and not misbranded. A single advisory review panel shall be established for each designated category of OTC drugs and every OTC drug category will be considered by a panel. The members of a panel shall be qualified experts (appointed by the Commissioner) and may include persons from lists submitted by organizations representing professional, consumer, and industry interests. The Commissioner shall designate the chairman of each panel. Summary minutes of all meetings shall be made.

(2) *Request for data and views.* The Commissioner will publish a notice in the FEDERAL REGISTER requesting interested persons to submit, for review and evaluation by an advisory review panel, published and unpublished data and information pertinent to a designated category of OTC drugs. Data and information submitted pursuant to a published notice, and falling within the confidentiality provisions of 18 U.S.C. 1905, 5 U.S.C. 552(b), or 21 U.S.C. 331(j), shall be handled by the advisory review panel and the Food and Drug Administration as confidential until publication of a proposed monograph and the full report(s) of the panel. Thirty days thereafter such data and information shall be made publicly available and may be viewed at the office of the Dockets Management Branch of the Food and Drug Administration, except to the extent that the person submitting it demonstrates that it still falls within the confidentiality provisions of one or more of those statutes. To be considered, eight copies of the data and/or views on any marketed drug within the class must be submitted, preferably bound, indexed, and on standard sized paper (approximately 8½ x 11 inches). When requested, abbreviated submissions should be sent. All submissions must be in the following format:

OTC DRUG REVIEW INFORMATION

I. Label(s) and all labeling (preferably mounted and filed with the other data—facsimile labeling is acceptable in lieu of actual container labeling).
II. A statement setting forth the quantities of active ingredients of the drug.
III. Animal safety data.
A. Individual active components.
1. Controlled studies.

2. Partially controlled or uncontrolled studies.
B. Combinations of the individual active components.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
C. Finished drug product.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
IV. Human safety data.
A. Individual active components.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination as to the safety of each individual active component.
5. Pertinent medical and scientific literature.
B. Combinations of the individual active components.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination as to the safety of combinations of the individual active components.
5. Pertinent medical and scientific literature.
C. Finished drug product.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination as to the safety of the finished drug product.
5. Pertinent medical and scientific literature.
V. Efficacy data.
A. Individual active components.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination on the efficacy of each individual active component.
5. Pertinent medical and scientific literature.
B. Combinations of the individual active components.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination on the efficacy of combinations of the individual active components.
5. Pertinent medical and scientific literature.
C. Finished drug product.
1. Controlled studies.
2. Partially controlled or uncontrolled studies.
3. Documented case reports.
4. Pertinent marketing experiences that may influence a determination on the efficacy of the finished drug product.
5. Pertinent medical and scientific literature.
VI. A summary of the data and views setting forth the medical rationale and purpose (or lack thereof) for the drug and its ingredients and the scientific basis (or lack thereof) for the conclusion that the drug and its ingredients have been proven safe and effective for the intended use. If there is an absence of controlled studies in the material submitted, an explanation as to why such studies are not considered necessary must be included.

(3) *Deliberations of an advisory review panel.* An advisory review panel will meet as often and for as long as is appropriate to review the data submitted to it and to prepare a report containing its conclusions and recommendations to the Commissioner with respect to the safety and effectiveness of the drugs in a designated category of OTC drugs. A panel may consult any individual or group. Any interested person may request an opportunity to present oral views to the panel; such request may be granted or denied by the panel. Such requests for oral presentations should be in written form including a summarization of the data to be presented to the panel. Any interested person may present written data and views which shall be considered by the panel. This information shall be presented to the panel in the format set forth in paragraph (a)(2) of this section and within the time period established for the drug category in the notice for review by a panel.

(4) *Standards for safety, effectiveness, and labeling.* The advisory review panel, in reviewing the data submitted to it and preparing its conclusions and recommendations, and the Commissioner, in reviewing the conclusions and recommendations of the panel and the published proposed, tentative, and the final monographs, shall apply the following standards to determine general recognition that a category of OTC drugs is safe and effective and not misbranded:

(i) Safety means a low incidence of adverse reactions or significant side effects under adequate directions for use and warnings against unsafe use as well as low potential for harm which may result from abuse under conditions of widespread availability. Proof of safety shall consist of adequate tests by methods reasonably applicable to show the drug is safe under the prescribed, recommended, or suggested conditions of use. This proof shall include results of significant human experience during marketing. General recognition of safety shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data.

(ii) Effectiveness means a reasonable expectation that, in a significant proportion of the target population, the pharmacological effect of the drug, when used under adequate directions for use and warnings against unsafe use, will provide clinically significant relief of the type claimed. Proof of effectiveness shall consist of controlled clinical investigations as defined in § 314.126(b) of this chapter, unless this requirement is waived on the basis of a showing that it is not reasonably applicable to the drug or essential to the validity of the investigation and that an alternative method of investigation is adequate to substantiate effectiveness. Investigations may be corroborated by partially controlled or uncontrolled studies, documented clinical studies by qualified experts, and reports of significant human experience during marketing. Isolated case reports, random experience, and reports lacking the details which permit scientific evaluation will not be considered. General recognition of effectiveness shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data.

(iii) The benefit-to-risk ratio of a drug shall be considered in determining safety and effectiveness.

(iv) An OTC drug may combine two or more safe and effective active ingredients and may be generally recognized as safe and effective when each active ingredient makes a contribution to the claimed effect(s); when combining of the active ingredients does not decrease the safety or effectiveness of any of the individual active ingredients; and when the combination, when used under adequate directions for use and warnings against unsafe use, provides rational concurrent therapy for a significant proportion of the target population.

(v) Labeling shall be clear and truthful in all respects and may not be false or misleading in any particular. It shall state the intended uses and results of the product; adequate directions for proper use; and warnings against unsafe use, side effects, and adverse reactions in such terms as to render them likely to be read and understood by the ordinary individual, including individuals of low comprehension, under customary conditions of purchase and use.

(vi) A drug shall be permitted for OTC sale and use by the laity unless, because of its toxicity or other potential for harmful effect or because of the method or collateral measures necessary to its use, it may safely be sold and used only under the supervision of a practitioner licensed by law to administer such drugs.

(5) *Advisory review panel report to the Commissioner.* An advisory review panel shall submit to the Commissioner a report containing its conclusions and recommendations with respect to the conditions under which OTC drugs falling within the category covered by the panel are generally recognized as safe and effective and not misbranded. Included within this report shall be:

(i) A recommended monograph or monographs covering the category of OTC drugs and establishing conditions under which the drugs involved are generally recognized as safe and effective and not misbranded (Category I). This monograph may include any conditions relating to active ingredients, labeling indications, warnings and adequate directions for use, prescription or OTC status, and any other conditions necessary and appropriate for the safety and effectiveness of drugs covered by the monograph.

(ii) A statement of all active ingredients, labeling claims or other statements, or other conditions reviewed and excluded from the monograph on the basis of the panel's determination that they would result in the drug's

not being generally recognized as safe and effective or would result in misbranding (Category II).

(iii) A statement of all active ingredients, labeling claims or other statements, or other conditions reviewed and excluded from the monograph on the basis of the panel's determination that the available data are insufficient to classify such condition under either paragraph (a)(5) (i) or (ii) of this section and for which further testing is therefore required (Category III). The report may recommend the type of further testing required and the time period within which it might reasonably be concluded.

(6) *Proposed monograph.* After reviewing the conclusions and recommendations of the advisory review panel, the Commissioner shall publish in the FEDERAL REGISTER a proposed order containing:

(i) A monograph or monographs establishing conditions under which a category of OTC drugs is generally recognized as safe and effective and not misbranded (Category I).

(ii) A statement of the conditions excluded from the monograph on the basis of the Commissioner's determination that they would result in the drug's not being generally recognized as safe and effective or would result in misbranding (Category II).

(iii) A statement of the conditions excluded from the monograph on the basis of the Commissioner's determination that the available data are insufficient to classify such conditions under either paragraph (a)(6)(i) or (ii) of this section (Category III).

(iv) The full report(s) of the panel to the Commissioner. The proposed order shall specify a reasonable period of time within which conditions falling within paragraph (a)(6)(iii) of this section may be continued in marketed products while the data necessary to support them are being obtained for evaluation by the Food and Drug Administration. The summary minutes of the panel meetings shall be made available to interested persons upon request. Any interested person may, within 90 days after publication of the proposed order in the FEDERAL REGISTER, file with the Dockets Management Branch of the Food and Drug Administration written comments in quintuplicate. Comments may be accompanied by a memorandum or brief in support thereof. All comments may be reviewed at the office of the Dockets Management Branch during regular working hours, Monday through Friday. Within 30 days after the final day for submission of comments, reply comments may be filed with the Dockets Management Branch; these comments shall be utilized to reply to comments made by other interested persons and not to reiterate a position. The Commissioner may satisfy this requirement by publishing in the FEDERAL REGISTER a proposed order summarizing the full report of the advisory review panel, containing its conclusions and recommendations, to obtain full public comment before undertaking his own evaluation and decision on the matters involved.

(7) *Tentative final monograph.* (i) After reviewing all comments, reply comments, and any new data and information, the Commissioner shall publish in the FEDERAL REGISTER a tentative order containing a monograph establishing conditions under which a category of OTC drugs is generally recognized as safe and effective and not misbranded. Within 60 days, any interested person may file with the Dockets Management Branch, Food and Drug Administration, written comments or written objections specifying with particularity the omissions or additions requested. These objections are to be supported by a brief statement of the grounds therefor. A request for an oral hearing may accompany such objections.

(ii) The Commissioner may publish in the FEDERAL REGISTER a separate tentative order containing a statement of those active ingredients reviewed and proposed to be excluded from the monograph on the basis of the Commissioner's determination that they would result in a drug product not being generally recognized as safe and effective or would result in misbranding, and for which no substantive comments in opposition to the panel report or new data and information were received by the Food and Drug Administration pursuant to paragraph (a)(6)(iv) of this section. Within 60 days, any interested

person may file with the Dockets Management Branch, Food and Drug Administration, written objections specifying with particularity the provision of the tentative order to which objection is made. These objections are to be supported by a brief statement of the grounds therefor. A request for an oral hearing may accompany such objections.

(iii) Within 12 months after publishing a tentative order pursuant to paragraph (a)(7)(i) of this section, any interested person may file with the Dockets Management Branch, Food and Drug Administration, new data and information to support a condition excluded from the monograph in the tentative order.

(iv) Within 60 days after the final day for submission of new data and information, comments on the new data and information may be filed with the Dockets Management Branch, Food and Drug Administration.

(v) New data and information submitted after the time specified in this paragraph but prior to the establishment of a final monograph will be considered as a petition to amend the monograph and will be considered by the Commissioner only after a final monograph has been published in the FEDERAL REGISTER unless the Commisisoner finds that good cause has been shown that warrants earlier consideration.

(8) *Oral hearing before the Commissioner.* After reviewing objections filed in response to the tentative final monograph, the Commissioner, if he finds reasonable grounds in support thereof, shall by notice in the FEDERAL REGISTER schedule an oral hearing. The notice scheduling an oral hearing shall specify the length of the hearing and how the time shall be divided among the parties requesting the hearing. The hearing shall be conducted by the Commissioner and may not be delegated.

(9) *Final monograph.* After reviewing the objections, the entire administrative record including all new data and information and comments, and considering the arguments made at any oral hearing, the Commissioner shall publish in the FEDERAL REGISTER a final order containing a monograph establishing conditions under which a category of OTC drugs is generally recognized as safe and effective and not misbranded. The monograph shall become effective as specified in the order.

(10) *Administrative record.* (i) All data and information to be considered in any proceeding pursuant to this section shall be submitted in response to the request for data and views pursuant to paragraph (a)(2) of this section or accepted by the panel during its deliberations pursuant to paragraph (a)(3) of this section or submitted to the Dockets Management Branch as part of the comments during the 90-day period and 30-day rebuttal comment period permitted pursuant to paragraph (a)(6) of this section or submitted to the Dockets Management Branch during the 12-month period or as part of the comments during the 60-day period permitted pursuant to paragraph (a)(7) of this section.

(ii) The Commissioner shall make all decisions and issue all orders pursuant to this section solely on the basis of the administrative record, and shall not consider data or information not included as part of the administrative record.

(iii) The administrative record shall consist solely of the following material: All notices and orders published in the FEDERAL REGISTER, all data and views submitted in response to the request published pursuant to paragraph (a)(2) of this section or accepted by the panel during its deliberations pursuant to paragraph (a)(3) of this section, all minutes of panel meetings, the panel report(s), all comments and rebuttal comments submitted on the proposed monograph and all new data and information submitted pursuant to paragraph (a)(6) of this section, all objections submitted on the tentative final monograph and all new data and information and comments submitted pursuant to paragraph (a)(7) of this section, the complete record of any oral public hearing conducted pursuant to paragraph (a)(8) of this section, all other comments requested at any time by the Commissioner, all data and information for which the Commissioner has reopened the administrative record, and all other material that the

Commissioner includes in the administrative record as part of the basis for the Commissioner's decision.

(11) *Court appeal.* The monograph contained in the final order constitutes final agency action from which appeal lies to the courts. The Food and Drug Administration will request consolidation of all appeals in a single court. Upon court appeal, the Commissioner may, at his discretion, stay the effective date for part or all of the monograph pending appeal and final court adjudication.

(12) *Amendment of monographs.* (i) The Commissioner may propose on the Commissioner's own initiative to amend or repeal any monograph established pursuant to this section. Any interested person may petition the Commissioner for such proposal pursuant to § 10.30 of this chapter. The Commissioner may deny the petition if the Commissioner finds a lack of safety or effectiveness employing the standards in paragraph (a)(4) of this section (in which case the appeal provisions of paragraph (a)(11) of this section shall apply), or the Commissioner may publish a proposed amendment or repeal in the FEDERAL REGISTER if the Commissioner finds general recognition of safety and effectiveness employing the standards in paragraph (a)(4) of this section. Any interested person may, within 60 days after publication of the proposed order in the FEDERAL REGISTER, file with the Dockets Management Branch, Food and Drug Administration, written comments in quadruplicate. Comments may be accompanied by a memorandum or brief in support thereof. All comments may be reviewed in the Dockets Management Branch between the hours of 9 a.m. and 4 p.m., Monday through Friday. After reviewing the comments, the Commissioner shall publish a final order amending the monograph established under the provisions of paragraph (a)(9) of this section or withdraw the proposal if comments opposing the amendment are persuasive. A new drug application may be submitted in lieu of, or in addition to, a petition under this paragraph.

(ii) A new drug application may be submitted in lieu of a petition to amend the OTC drug monograh only if the drug product with the condition that is the subject of the new drug application has not been marketed on an interim basis (such as under the provisions of paragraph (a)(6)(iii) of this section), all clinical testing has been conducted pursuant to a new drug application plan, and no marketing of the product with the condition for which approval is sought is undertaken prior to approval of the new drug application. The Food and Drug Administration shall handle a new drug application as a petition for amendment of a monograph, and shall review it on that basis, if the provisions of this paragraph preclude approval of a new drug application but permit the granting of such a petition.

(b) *Regulatory action.* Any product which fails to conform to an applicable monograph after its effective date is liable to regulatory action.

(c) Information and data submitted under this section shall include, with respect to each nonclinical laboratory study contained in the application, either a statement that the study was conducted in compliance with the good laboratory practice regulations set forth in part 58 of this chapter, or, if the study was not conducted in compliance with such regulations, a brief statement of the reason for the noncompliance.

(d) [Reserved]

(e) *Institutional review and informed consent.* Information and data submitted under this section after July 27, 1981, shall include statements regarding each clinical investigation involving human subjects, from which the information and data are derived, that it either was conducted in compliance with the requirements for institutional review set forth in part 56 of this chapter, or was not subject to such requirements in accordance with §§ 56.104 or 56.105, and that it was conducted in compliance with the requirements for informed consent set forth in part 50 of this chapter.

(f) *Financial certification or disclosure statement.* Any clinical data submitted under this section must be accompanied by financial certifications or

disclosure statements or both as required by part 54 of this chapter.

[39 FR 11741, Mar. 29, 1974, as amended at 39 FR 39556, Nov. 8, 1974; 42 FR 19141, Apr. 12, 1977; 42 FR 54800, Oct. 11, 1977; 46 FR 8460, 8955, Jan. 27, 1981; 46 FR 14340, Feb. 27, 1981; 46 FR 21360, Apr. 10, 1981; 46 FR 47738, Sept. 29, 1981; 50 FR 7516, Feb. 22, 1985; 55 FR 11581, Mar. 29, 1990; 63 FR 5253, Feb. 2, 1998]

EFFECTIVE DATE NOTE: At 63 FR 5253, Feb. 2, 1998, § 330.10 was amended by adding new paragraph (f), effective Feb. 2, 1999.

§ 330.11 NDA deviations from applicable monograph.

A new drug application requesting approval of an OTC drug deviating in any respect from a monograph that has become final shall be in the form required by § 314.50 of this chapter, but shall include a statement that the product meets all conditions of the applicable monograph except for the deviation for which approval is requested and may omit all information except that pertinent to the deviation.

[39 FR 11741, Mar. 29, 1974, as amended at 55 FR 11581, Mar. 29, 1990]

§ 330.12 Status of over-the-counter (OTC) drugs previously reviewed under the Drug Efficacy Study (DESI).

(a) There were 420 OTC drugs reviewed in the Drug Efficacy Study (a review of drugs introduced to the market through new drug procedures between 1938 and 1962). A careful review has been made of the reports on these drugs to determine those drugs for which implementation may be deferred without significant risk to the public health, pending review by appropriate OTC drug advisory review panels and promulgation of a monograph.

(b) On and after April 20, 1972, a number of notices were published in the FEDERAL REGISTER concerning previously unpublished OTC drugs reviewed by the National Academy of Sciences-National Research Council Drug Efficacy Study Group. Only the evaluations and comments of the panels were published, with no conclusions of the Commissioner of Food and Drugs. Those publications were for the purpose of giving interested persons the benefit of the Academy's opinions. For those products, and also for OTC drug products previously published with the Commissioner's conclusions (except for the products listed in paragraphs (b) (1) and (2) of this section, all requests for data, revised labeling, requests for new drug applications, abbreviated new drug applications, updating supplements, data to support less than effective claims, if any, etc., are deferred, and such OTC drug products are instead subject to the OTC drug review in their appropriate classes pursuant to the procedures established in this subpart.

(1) The requirements of the following DESI announcements are not deferred (the reference document may also pertain to prescription drugs):

(i) Certain Surgical Sutures (DESI 4725), published in the FEDERAL REGISTER of November 11, l971 (36 FR 21612).

(ii) Absorbable Dusting Powder (DESI 6264), published in the FEDERAL REGISTER of May 25, 1971 (36 FR 9475).

(iii) Certain Insulin Preparations (DESI 4286), published in the FEDERAL REGISTER of April 9, 1971 (36 FR 6842).

(iv) Sulfo-Van Ointment (DESI 2230), published in the FEDERAL REGISTER of October 8, 1970 (35 FR 15860).

(v) Antiperspirants and Deodorants Containing Neomycin Sulfate (DESI 11048) for which an order revoking provisions for certification or release was published in the FEDERAL REGISTER of December 5, 1972 (37 FR 25820) and has been stayed by the filing of objections.

(vi) Thorexin Cough Medicine (DESI 11160) for which a notice of opportunity for hearing was published in the FEDERAL REGISTER of February 2, 1973 (38 FR 3210).

(vii) Antibiotic susceptibility discs (DESI 90235) for which an order providing for certain discs to be certified and removing provisions for certification of other discs was published in the FEDERAL REGISTER of September 30, 1972 (37 FR 20525) and has been stayed by the filing of objections notice of which was published in the FEDERAL REGISTER of March 15, 1973 (38 FR 7007).

(2) Deferral of requirements is not appropriate when an announcement has been published and has been followed by a final order classifying a drug either as lacking substantial evidence of effectiveness or as not shown to be safe. These products will be removed

from the market, if they have not already been removed. Regulatory action will also be undertaken against identical, similar and related products (21 CFR 310.6). Deferral of requirements is not appropriate for the following (the referenced document may also pertain to prescription drugs):

(i) Certain Sulfonamide-Decongestant Nasal Preparation (DESI 4850), for which notice of withdrawal of approval of new drug applications was published in the FEDERAL REGISTER of October 24, 1970 (35 FR 16605, 16606).

(ii) Eskay's Theranates, containing strychnine, sodium, and calcium glycerophosphates, thiamine hydrochloride, alcohol, and phosphoric acid (DESI 2220), for which notice of withdrawal of approval of the new drug application was published in the FEDERAL REGISTER of February 18, 1971 (36 FR 3152).

(iii) The following topical drugs (DESI 1726), for which notice of withdrawal of new drug applications was published in the FEDERAL REGISTER of August 28, 1971 (36 FR 17368):

(*a*) Rhulitol Solution, containing tannic acid, chlorobutanol, phenol, camphor, alum, and isopropyl alcohol.

(*b*) Zirnox Topical Lotion, containing phenyitoloxamine citrate and zirconium oxide.

(iv) Menacyl Tablets, containing aspirin, menadione, and ascorbic acid (DESI 6363), for which notice of withdrawal of approval of the new drug application was published in the FEDERAL REGISTER of July 23, 1970 (35 FR 11827).

(v) Curad Medicated Adhesive Bandage containing sulfathiazole (DESI 4964), for which notice of withdrawal of approval of the new drug application was published in the FEDERAL REGISTER of December 31, 1969 (34 FR 20441).

(vi) Drugs Containing Rutin, Quercetin, Hesperidin, or any Bioflavonoids (DESI 5960), for which notice of withdrawal of approval of new drug applications was published in the FEDERAL REGISTER of July 3, 1970 (35 FR 10872, 10873) and October 17, 1970 (35 FR 16332). A further notice of opportunity for hearing with respect to the drugs covered by the October 17, 1970 FEDERAL REGISTER notice will be published at a later date.

(vii) Antibiotics in Combination with Other Drugs for Nasal Use (DESI 7561), for which an order revoking provision for certification was published in the FEDERAL REGISTER of August 6, 1971 (36 FR 14469) and confirmed in the FEDERAL REGISTER of October 28, 1971 (36 FR 20686).

(viii) Antibiotic Troches (DESI 8328), for which an order revoking provision for certification was published in the FEDERAL REGISTER of July 14, 1971 (36 FR 13089) and confirmed in the FEDERAL REGISTER of October 9, 1971 (36 FR 19695).

(ix) Certain Drugs Containing Oxyphenisatin or Oxyphenisatin Acetate (DESI 10732), for which notices of withdrawal of approval of new drug applications were published in the FEDERAL REGISTER of February 1, 1972 (37 FR 2460), and March 9, 1973 (38 FR 6419).

(x) Curad Medicated Adhesive Bandage containing tyrothricin-nitrofurazone (DESI 6898), for which an order revoking provision for certification was published March 14, 1972 (37 FR 5294), and confirmed in the FEDERAL REGISTER of July 6, 1972 (37 FR 13254).

(xi) Candette Cough Gel (DESI 11562), for which notice of withdrawal of approval of the new drug application was published in the FEDERAL REGISTER of November 19, 1972 (37 FR 25249).

(xii) Certain OTC Multiple-Vitamin Preparations for Oral Use containing excessive amounts of vitamin D and/or vitamin A (DESI 97), for which notice of withdrawal of approval of the new drug applications was published in the FEDERAL REGISTER of November 29, 1972 (37 FR 25249).

(xiii) Certain Sulfonamide-Containing Preparations for Topical Ophthalmic or Otic Use (DESI 368, for which a notice of withdrawal of approval was published in the FEDERAL REGISTER of February 2, 1973 (38 FR 3208).

(xiv) Those parts of the publication entitled ''Certain Mouthwash and Gargle Preparations'' (DESI 2855) pertaining to Tyrolaris Mouthwash, containing tyrothricin, panthenol, and alcohol, for which an order revoking provision for certification was published in the FEDERAL REGISTER of February 2, 1967 (32 FR 1172) prior to the drug efficacy study implementation.

(c) Manufacturers and distributors should take notice that the information on OTC drugs provided by the Drug Efficacy Study review is valuable information as to the deficiencies in the data available to support indications for use. They are encouraged to perform studies to obtain adequate evidence of effectiveness for the review of OTC drugs which is already in progress. In the interim it is in the public interest that manufacturers and distributors of all OTC drugs effect changes in their formulations and/or labeling to bring the products into conformity with current medical knowledge and experience.

(d) Manufacturers and distributors of OTC drugs may be reluctant to make appropriate formulation and/or labeling changes for fear of losing the protection of the so-called ''grandfather'' provisions of the 1938 Federal Food, Drug, and Cosmetic Act (sec. 201(p)(1)) and the 1962 amendments to the act (sec. 107(c) of those amendments). To encourage and facilitate prompt changes, the Food and Drug Administration will not take legal action against any OTC drug, other than those not deferred, based on a charge that the product is a new drug and not grandfathered under the act as a result of the changes if the changes in formulation and/or labeling are of the following kind:

(1) The addition to the labeling of warning, contraindications, side effects, and/or precaution information.

(2) The deletion from the labeling of false, misleading, or unsupported indications for use or claims of effectiveness.

(3) Changes in the components or composition of the drug that will give increased assurance that the drug will have its intended effect, yet not raise or contribute any added safety questions.

(4) Changes in the components or composition of the drug which may reasonably be concluded to improve the safety of the drug, without diminishing its effectiveness.

(e) The forbearance from legal action for lack of grandfather protection is an interim procedure designed to encourage appropriate change in formulation and/or labeling during the time period required to review the various classes of OTC drugs. At such time as an applicable OTC drug monograph becomes effective, the interim procedure will automatically be terminated and any appropriate regulatory action will be initiated.

§ 330.13 Conditions for marketing ingredients recommended for over-the-counter (OTC) use under the OTC drug review.

(a) Before the publication in the FEDERAL REGISTER of an applicable proposed monograph, an OTC drug product that contains: (1) An active ingredient limited, on or after May 11, 1972, to prescription use for the indication and route of administration under consideration by an OTC advisory review panel, and not thereafter exempted from such limitation pursuant to § 310.200 of this chapter, or

(2) An active ingredient at a dosage level higher than that available in an OTC drug product on December 4, 1975, shall be regarded as a new drug within the meaning of section 201(p) of the act for which an approved new drug application is required.

(b)(1) An OTC drug product that contains: (i) An active ingredient limited, on or after May 11, 1972, to prescription use for the indication and route of administration under consideration by an OTC advisory review panel, and not thereafter exempted from such limitation pursuant to § 310.200 of this chapter, or

(ii) An active ingredient at a dosage level higher than that available in an OTC drug product on December 4, 1975, which ingredient and/or dosage level is classified by the panel in category I (conditions subject to § 330.10(a)(6)(i)) shall be regarded as a new drug within the meaning of section 201(p) of the act for which an approved new drug application is required if marketed for OTC use prior to the date of publication in the FEDERAL REGISTER of a proposed monograph.

(2) An OTC drug product covered by paragraph (b)(1) of this section which is marketed after the date of publication in the FEDERAL REGISTER of a proposed monograph but prior to the effective date of a final monograph shall be subject to the risk that the Commissioner

may not accept the panel's recommendation and may instead adopt a different position that may require relabeling, recall, or other regulatory action. The Commissioner may state such position at any time by notice in the FEDERAL REGISTER, either separately or as part of another document; appropriate regulatory action will commence immediately and will not await publication of a final monograph. Marketing of such a product with a formulation or labeling not in accord with a proposed monograph or tentative final monograph also may result in regulatory action against the product, the marketer, or both.

(c) An OTC drug product that contains: (1) An active ingredient limited, on or after May 11, 1972, to prescription use for the indication and route of administration under consideration by an OTC advisory review panel, and not thereafter exempted from such limitation pursuant to §310.200 of this chapter, or

(2) An active ingredient at a dosage level higher than that available in any OTC drug product on December 4, 1975, which ingredient and/or dosage level is classified by the panel in category II (conditions subject to §330.10(a)(6)(ii)), may be marketed only after:

(i) The Center for Drug Evaluation and Research or the Commissioner tentatively determines that the ingredient is generally recognized as safe and effective, and the Commissioner states by notice in the FEDERAL REGISTER (separately or as part of another document) that marketing under specified conditions will be permitted;

(ii) The ingredient is determined by the Commissioner to be generally recognized as safe and effective and is included in the appropriate published OTC drug final monograph; or

(iii) A new drug application for the product has been approved.

(d) An OTC drug product that contains: (1) An active ingredient limited, on or after May 11, 1972, to prescription use for the indication and route of administration under consideration by an OTC advisory review panel, and not thereafter exempted from such limitation pursuant to §310.200 of this chapter, or

(2) An active ingredient at a dosage level higher than that available in any OTC drug product on December 4, 1975, which ingredient and/or dosage level is classified by the panel in category III (conditions subject to §330.10(a)(6)(iii)), may be marketed only after:

(i) The Center for Drug Evaluation and Research or the Commissioner tentatively determines that the ingredient is generally recognized as safe and effective, and the Commissioner states by notice in the FEDERAL REGISTER (separately or as part of another document) that marketing under specified conditions will be permitted;

(ii) The ingredient is determined by the Commissioner to be generally recognized as safe and effective and is included in the appropriate published OTC drug final monograph; or

(iii) A new drug application for the product has been approved.

[41 FR 32582, Aug. 4, 1976, as amended at 47 FR 17739, Apr. 23, 1982; 50 FR 8996, Mar. 6, 1985; 55 FR 11581, Mar. 29, 1990]

PART 331—ANTACID PRODUCTS FOR OVER-THE-COUNTER (OTC) HUMAN USE

Subpart A—General Provisions

Sec.
331.1 Scope.

Subpart B—Active Ingredients

331.10 Antacid active ingredients.
331.11 Listing of specific active ingredients.
331.15 Combination with nonantacid active ingredients.

Subpart C—Testing Procedures

331.20 Determination of percent contribution of active ingredients.
331.21 Test Modifications.

Subpart D—Labeling

331.30 Labeling of antacid products.
331.80 Professional labeling.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 39 FR 19874, June 4, 1974, unless otherwise noted.

Subpart A—General Provisions

§ 331.1 Scope.

An over-the-counter antacid product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each of the following conditions and each of the general conditions established in § 330.1 of this chapter.

Subpart B—Active Ingredients

§ 331.10 Antacid active ingredients.

(a) The active antacid ingredients of the product consist of one or more of the ingredients permitted in § 331.11 within any maximum daily dosage limit established, each ingredient is included at a level that contributes at least 25 percent of the total acid neutralizing capacity of the product, and the finished product contains at least 5 meq of acid neutralizing capacity as measured by the procedure provided in the United States Pharmacopeia 23/National Formulary 18. The method established in § 331.20 shall be used to determine the percent contribution of each antacid active ingredient.

(b) This section does not apply to an antacid ingredient specifically added as a corrective to prevent a laxative or constipating effect.

[39 FR 19874, June 4, 1974, as amended at 61 FR 4822, Feb. 8, 1996]

§ 331.11 Listing of specific active ingredients.

(a) Aluminum-containing active ingredients:

(1) Basic aluminum carbonate gel.

(2) Aluminum hydroxide (or as aluminum hydroxide-hexitol stabilized polymer, aluminum hydroxide-magnesium carbonate codried gel, aluminum hydroxide-magnesium trisilicate codried gel, aluminum-hydroxide sucrose powder hydrated).

(3) Dihydroxyaluminum aminoacetate and dihydroxyaluminum aminoacetic acid.

(4) Aluminum phosphate gel when used as part of an antacid combination product and contributing at least 25 percent of the total acid neutralizing capacity; maximum daily dosage limit is 8 grams.

(5) Dihydroxyaluminum sodium carbonate.

(b) Bicarbonate-containing active ingredients: Bicarbonate ion; maximum daily dosage limit 200 mEq. for persons up to 60 years old and 100 mEq. for persons 60 years or older.

(c) Bismuth-containing active ingredients:

(1) Bismuth aluminate.

(2) Bismuth carbonate.

(3) Bismuth subcarbonate.

(4) Bismuth subgallate.

(5) Bismuth subnitrate.

(d) Calcium-containing active ingredients: Calcium, as carbonate or phosphate; maximum daily dosage limit 160 mEq. calcium (e.g., 8 grams calcium carbonate).

(e) Citrate-containing active ingredients: Citrate ion, as citric acid or salt; maximum daily dosage limit 8 grams.

(f) Glycine (aminoacetic acid).

(g) Magnesium-containing active ingredients:

(1) Hydrate magnesium aluminate activated sulfate.

(2) Magaldrate.

(3) Magnesium aluminosilicates.

(4) Magnesium carbonate.

(5) Magnesium glycinate.

(6) Magnesium hydroxide.

(7) Magnesium oxide.

(8) Magnesium trisilicate.

(h) Milk solids, dried.

(i) Phosphate-containing active ingredients:

(1) Aluminum phosphate; maximum daily dosage limit 8 grams.

(2) Mono or dibasic calcium salt; maximum daily dosage limit 2 grams.

(3) Tricalcium phosphate; maximum daily dosage limit 24 grams.

(j) Potassium-containing active ingredients:

(1) Potassium bicarbonate (or carbonate when used as a component of an effervescent preparation); maximum daily dosage limit 200 mEq. of bicarbonate ion for persons up to 60 years old and 100 mEq. of bicarbonate ion for persons 60 years or older.

(2) Sodium potassium tartrate.

(k) Sodium-containing active ingredients:

(1) Sodium bicarbonate (or carbonate when used as a component of an effervescent preparation); maximum daily dosage limit 200 mEq. of sodium for

persons up to 60 years old and 100 mEq. of sodium for persons 60 years or older, and 200 mEq. of bicarbonate ion for persons up to 60 years old and 100 mEq. of bicarbonate ion for persons 60 years or older. That part of the warning required by § 330.1(g), which states, ''Keep this and all drugs out of the reach of children'' is not required on a product which contains only sodium bicarbonate powder and which is intended primarily for other than drug uses.

(2) Sodium potassium tartrate.

(l) Silicates:

(1) Magnesium aluminosilicates.

(2) Magnesium trisilicate.

(m) Tartrate-containing active ingredients. Tartaric acid or its salts; maximum daily dosage limit 200 mEq. (15 grams) of tartrate.

[39 FR 19874, June 4, 1974, as amended at 51 FR 27763, Aug. 1, 1986; 55 FR 19859, May 11, 1990]

§ 331.15 Combination with nonantacid active ingredients.

(a) An antacid may contain any generally recognized as safe and effective nonantacid laxative ingredient to correct for constipation caused by the antacid. No labeling claim of the laxative effect may be used for such a product.

(b) An antacid may contain any generally recognized as safe and effective analgesic ingredient(s), if it is indicated for use solely for the concurrent symptoms involved, e.g., headache and acid indigestion, and is marketed in a form intended for ingestion as a solution.

(c) An antacid may contain any generally recognized as safe and effective antiflatulent ingredient if it is indicated for use solely for the concurrent symptoms of gas associated with heartburn, sour stomach or acid indigestion.

Subpart C—Testing Procedures

§ 331.20 Determination of percent contribution of active ingredients.

To determine the percent contribution of an antacid active ingredient, place an accurately weighed amount of the antacid active ingredient equal to the amount present in a unit dose of the product into a 250-milliliter (mL) beaker. If wetting is desired, add not more than 5 mL of alcohol (neutralized to an apparent pH of 3.5), and mix to wet the sample thoroughly. Add 70 mL of water, and mix on a magnetic stirrer at 300±30 r.p.m. for 1 minute. Analyze the acid neutralizing capacity of the sample according to the procedure provided in the United States Pharmacopeia 23/National Formulary 18 and calculate the percent contribution of the antacid active ingredient in the total product as follows:

Percent contribution = (Total mEq. Antacid Active Ingredient x100)/(Total mEq. Antacid Product).

[61 FR 4823, Feb. 8, 1996]

§ 331.21 Test modifications.

The formulation or mode of administration of certain products may require a modification of the United States Pharmacopeia 23/National Formulary 18 acid neutralizing capacity test. Any proposed modification and the data to support it shall be submitted as a petition under the rules established in § 10.30 of this chapter. All information submitted will be subject to the disclosure rules in part 20 of this chapter.

[61 FR 4823, Feb. 8, 1996]

Subpart D—Labeling

§ 331.30 Labeling of antacid products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antacid.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''For the relief of'' (optional, any or all of the following:) ''heartburn,'' ''sour stomach,'' and/or ''acid indigestion'' (which may be followed by the optional statement:) ''and upset stomach associated with'' (optional, as appropriate) ''this symptom'' or ''these symptoms.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction

into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings, under the heading ''Warnings'', which may be combined but not rearranged to eliminate duplicative words or phrases if the resulting warning is clear and understandable:

(1) ''Do not take more than (maximum recommended daily dosage, broken down by age groups if appropriate, expressed in units such as tablets or teaspoonfuls) in a 24–hour period, or use the maximum dosage of this product for more than 2 weeks, except under the advice and supervision of a physician.''

(2) For products which cause constipation in 5 percent or more of persons who take the maximum recommended dosage: ''May cause constipation.''

(3) For products which cause laxation in 5 percent or more of persons who take the maximum recommended dosage: ''May have laxative effect.''

(4) For products containing more than 50 mEq. of magnesium in the recommended daily dosage: ''Do not use this product except under the advice and supervision of a physician if you have kidney disease.''

(5) For products containing more than 25 mEq. potassium in the maximum recommended daily dose: ''Do not use this product except under the advice and supervision of a physician if you have kidney disease.''

(6) For products containing more than 5 gm per day lactose in a maximum daily dosage: ''Do not use this product except under advice and supervision of a physician if you are allergic to milk or milk products.''

(d) *Drug interaction precaution.* The labeling of the product contains the following statements under the heading ''Drug Interaction Precaution'': ''Antacids may interact with certain prescription drugs. If you are presently taking a prescription drug, do not take this product without checking with your physician or other health professional.''

(e) *Directions for use.* The labeling of the product contains the recommended dosage, under the heading ''Directions'', per time interval (e.g., every 4 hours) or time period (e.g., 4 times a day) broken down by age groups if appropriate, followed by ''or as directed by a physician.''

(f) *Exemption from the general accidental overdose warning.* The labeling for antacid drug products containing the active ingredients identified in § 331.11(a), (b), and (d) through (m); permitted combinations of these ingredients provided for in § 331.10; and any of these ingredients or combinations of these ingredients in combination with simethicone (identified in § 332.10 of this chapter and provided for in § 331.15(c)), are exempt from the requirement in § 330.1(g) of this chapter that the labeling bear the general warning statement ''In case of accidental overdose, seek professional assistance or contact a poison control center immediately.'' With the exception of sodium bicarbonate powder products identified in § 331.11(k)(1), the labeling must continue to bear the first part of the general warning in § 330.1(g) of this chapter, which states, ''Keep this and all drugs out of the reach of children.''

(g) [Reserved]

(h) The word ''doctor'' may be substituted for the word ''physician'' in any of the labeling statements in this section.

[39 FR 19874, June 4, 1974, as amended at 47 FR 38484, Aug. 31, 1982; 51 FR 16266, May 1, 1986; 51 FR 27763, Aug. 1, 1986; 52 FR 7830, Mar. 13, 1987; 55 FR 11581, Mar. 29, 1990; 58 FR 45208, Aug. 26, 1993; 59 FR 60556, Nov. 25, 1994; 61 FR 17806, Apr. 22, 1996]

§ 331.80 Professional labeling.

(a) The labeling of the product provided to health professionals (but not to the general public):

(1) Shall contain the neutralizing capacity of the product as calculated using the procedure set forth in United States Pharmacopeia 23/National Formulary 18 expressed in terms of the dosage recommended per minimum time interval or, if the labeling recommends more than one dosage, in terms of the minimum dosage recommended per minimum time interval.

(2) May contain an indication for the symptomatic relief of hyperacidity associated with the diagnosis of peptic

ulcer, gastritis, peptic esophagitis, gastric hyperacidity, and hiatal hernia.

(3) *For products containing basic aluminum carbonate gel identified in §331.11(a)(1)—Indication.* ''For the treatment, control, or management of hyperphosphatemia, or for use with a low phosphate diet to prevent formation of phosphate urinary stones, through the reduction of phosphates in the serum and urine.''

(4) *For products containing aluminum identified in §331.11(a)—Warnings.* (i) Prolonged use of aluminum-containing antacids in patients with renal failure may result in or worsen dialysis osteomalacia. Elevated tissue aluminum levels contribute to the development of the dialysis encephalopathy and osteomalacia syndromes. Small amounts of aluminum are absorbed from the gastrointestinal tract and renal excretion of aluminum is impaired in renal failure. Aluminum is not well removed by dialysis because it is bound to albumin and transferrin, which do not cross dialysis membranes. As a result, aluminum is deposited in bone, and dialysis osteomalacia may develop when large amounts of aluminum are ingested orally by patients with impaired renal function.

(ii) Aluminum forms insoluble complexes with phosphate in the gastrointestinal tract, thus decreasing phosphate absorption. Prolonged use of aluminum-containing antacids by normophosphatemic patients may result in hypophosphatemia if phosphate intake is not adequate. In its more severe forms, hypophosphatemia can lead to anorexia, malaise, muscle weakness, and osteomalacia.

(b) Professional labeling for an antacid-antiflatulent combination may contain the information allowed for health professionals for antacids and antiflatulents.

[39 FR 19874, June 4, 1974. Redesignated and amended at 55 FR 19859, May 11, 1990]

PART 332—ANTIFLATULENT PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
332.1 Scope.
332.3 Definitions.

Subpart B—Active Ingredients

332.10 Antiflatulent active ingredients.
332.15 Combination with non-antiflatulent active ingredients.

Subpart C—Labeling

332.30 Labeling of antiflatulent products.
332.31 Professional labeling.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 39 FR 19877, June 4, 1974, unless otherwise noted.

Subpart A—General Provisions

§ 332.1 Scope.

An over-the-counter antiflatulent product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each of the following conditions and each of the general conditions established in §330.1 of this chapter.

§ 332.3 Definitions.

As used in this part:

Antigas. A term that may be used interchangeably with the term antiflatulent. Neither term should be considered as describing the mechanism of action of the active ingredient contained in the product.

[61 FR 8838, Mar. 5, 1996]

Subpart B—Active Ingredients

§ 332.10 Antiflatulent active ingredients.

Simethicone; maximum daily dose 500 mg. There is no dosage limitation at this time for professional labeling.

§ 332.15 Combination with non-antiflatulent active ingredients.

An antiflatulent may contain any generally recognized as safe and effective antacid ingredient(s) if it is indicated for use solely for the concurrent symptoms of gas associated with heartburn, sour stomach or acid indigestion.

Subpart C—Labeling

§ 332.30 Labeling of antiflatulent drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antiflatulent,'' ''antigas,'' or ''antiflatulent (antigas).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' one or more of the phrases listed in this paragraph (b), as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) (Select one of the following: ''Alleviates or Relieves'') ''the symptoms referred to as gas.''

(2) (Select one of the following: ''Alleviates'' or ''Relieves'') (select one or more of the following: ''bloating,'' ''pressure,'' ''fullness,'' or ''stuffed feeling'') ''commonly referred to as gas.''

(c) *Exemption from the general accidental overdose warning.* The labeling for antiflatulent drug products containing simethicone identified in § 332.10 and antacid/antiflatulent combination drug products provided for in § 332.15, containing the active ingredients identified in § 331.11(a), (b), and (d) through (m) of this chapter are exempt from the requirement in § 330.1(g) of this chapter that the labeling bear the general warning statement ''In case of accidental overdose, seek professional assistance or contact a poison control center immediately.'' The labeling must continue to bear the first part of the general warning in § 330.1(g) of this chapter, which states, ''Keep this and all drugs out of the reach of children.''

[39 FR 19877, June 4, 1974, as amended at 40 FR 11719, Mar. 13, 1975; 51 FR 16266, May 1, 1986; 51 FR 27763, Aug. 1, 1986; 52 FR 7830, Mar. 13, 1987; 61 FR 8838, Mar. 5, 1996]

§ 332.31 Professional labeling.

(a) The labeling of the product provided to health professionals (but not to the general public) may contain as additional indications postoperative gas pain or for use in endoscopic examination.

(b) Professional labeling for an antiflatulent-antacid combination may contain information allowed for health professionals for antacids and antiflatulents.

PART 333—TOPICAL ANTIMICROBIAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A [Reserved]

Subpart B—First Aid Antibiotic Drug Products

Sec.
333.101 Scope.
333.103 Definitions.
333.110 First aid antibiotic active ingredients.
333.120 Permitted combinations of active ingredients.
333.150 Labeling of first aid antibiotic drug products.
333.160 Labeling of permitted combinations of active ingredients.

Subpart C—Topical Antifungal Drug Products

333.201 Scope.
333.203 Definitions.
333.210 Antifungal active ingredients.
333.250 Labeling of antifungal drug products.
333.280 Professional labeling.

Subpart D—Topical Acne Drug Products

333.301 Scope.
333.303 Definitions.
333.310 Acne active ingredients.
333.320 Permitted combinations of active ingredients.
333.350 Labeling of acne drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371, unless otherwise noted.

SOURCE: 52 FR 47322, Dec. 11, 1987, unless otherwise noted.

Subpart A—[Reserved]

Subpart B—First Aid Antibiotic Drug Products

§ 333.101 Scope.

(a) An over-the-counter first aid antibiotic drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart and each of the general conditions established in § 330.1.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 333.103 Definitions.

As used in this subpart:

(a) *Antibiotic drug.* In accordance with section 507(a) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357(a)), ''any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and which has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance).''

(b) *First aid antibiotic.* An antibiotic-containing drug product applied topically to the skin to help prevent infection in minor cuts, scrapes, and burns.

§ 333.110 First aid antibiotic active ingredients.

The product consists of any of the following active ingredients within the specified concentration established for each ingredient and in the specified dosage form:

(a) Bacitracin ointment containing, in each gram, 500 units of bacitracin in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.510a(b).

(b) Bacitracin zinc ointment containing, in each gram, 500 units of bacitracin zinc in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.513f(b).

(c) Chlortetracycline hydrochloride ointment containing, in each gram, 30 milligrams of chlortetracycline hydrochloride in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 446.510(b).

(d) Neomycin sulfate ointment containing, in each gram, 3.5 milligrams of neomycin in a suitable water soluble or oleaginous ointment base: *Provided,* That it meets the tests and methods of assay in § 444.542a(b).

(e) Neomycin sulfate cream containing, in each gram, 3.5 milligrams of neomycin in a suitable cream base: *Provided,* That it meets the tests and methods of assay in § 444.542b(b).

(f) Tetracycline hydrochloride ointment containing, in each gram, 30 milligrams of tetracycline hydrochloride in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 446.581d(b).

[52 FR 47322, Dec. 11, 1987, as amended at 53 FR 18838, May 25, 1988]

§ 333.120 Permitted combinations of active ingredients.

The following combinations are permitted provided each active ingredient is present within the established concentration and in the specified dosage form, and the product is labeled in accordance with § 333.160.

(a) *Combinations of antibiotic active ingredients.* (1) Bacitracin-neomycin sulfate ointment containing, in each gram, 500 units of bacitracin and 3.5 milligrams of neomycin in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.510d(b).

(2) Bacitracin-neomycin sulfate-polymyxin B sulfate ointment containing, in each gram, in a suitable ointment base the following:

(i) 500 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B;

Provided, That it meets the tests and methods of assay in § 448.510e(b).

(3) Bacitracin-polymyxin B sulfate topical aerosol containing, in each gram, 500 units of bacitracin and 5,000 units of polymyxin B in a suitable vehicle, packaged in a pressurized container with suitable inert gases: *Provided,* That it meets the tests and methods of assay in § 448.510f(b).

(4) Bacitracin zinc-neomycin sulfate ointment containing, in each gram, 500 units of bacitracin and 3.5 milligrams of neomycin in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.513b(b).

(5) Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ointment containing, in each gram, in a suitable ointment base the following:

(i) 400 units of bacitracin, 3 milligrams of neomycin, and 8,000 units of polymyxin B; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(iii) 500 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(iv) 500 units of bacitracin, 3.5 milligrams of neomycin, and 10,000 units of polymyxin B;

Provided, That it meets the tests and methods of assay in § 448.513c(b).

(6) Bacitracin zinc-polymyxin B sulfate ointment containing, in each gram, 500 units of bacitracin and 10,000 units of polymyxin B in a suitable ointment base: *Provided,* That it meets the tests and methods assay in § 448.513a(b).

(7) Bacitracin zinc-polymyxin B sulfate topical aerosol containing, in each gram, 120 units of bacitracin and 2,350 units of polymyxin B in a suitable vehicle, packaged in a pressurized container with suitable inert gases: *Provided,* That is meets the tests and methods of assay in § 448.513e(b) of this chapter.

(8) Bacitracin zinc-polymyxin B sulfate topical powder containing, in each gram, 500 units of bacitracin and 10,000 units of polymyxin B in a suitable base: *Provided,* That it meets the tests and methods of assay in § 448.513d(b).

(9) Neomycin sulfate-polymyxin B sulfate ointment containing, in each gram, 3.5 milligrams of neomycin and 5,000 units of polymyxin B in a suitable water miscible base: *Provided,* That it meets the tests and methods of assay in § 444.542e(b).

(10) Neomycin sulfate-polymyxin B sulfate cream containing, in each gram, 3.5 milligrams of neomycin and 10,000 units of polymyxin B in a suitable vehicle: *Provided,* That it meets the tests, methods of assay, and potency in § 444.5421(b).

(11) Oxytetracycline hydrochloride-polymyxin B sulfate ointment containing, in each gram, 30 milligrams of oxytetracycline and 10,000 units of polymyxin B in a suitable ointment base: *Provided,* That it meets the tests and methods assay in § 446.567b(b).

(12) Oxytetracycline hydrochloride-polymyxin B sulfate topical powder containing, in each gram, 30 milligrams of oxytetracycline and 10,000 units of polymyxin B with a suitable filler: *Provided,* That it meets the tests and methods assay in § 446.567c(b).

(b) *Combinations of first aid antibiotic active ingredients and local anesthetic active ingredients.*

(1) Bacitracin ointment containing, in each gram, 500 units of bacitracin and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.510a(b).

(2) Bacitracin-neomycin sulfate-polymyxin B sulfate ointment containing, in each gram, in a suitable ointment base the following:

(i) 500 units of bacitracin, 3.5 milligrams of neomycin, 5,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, 5,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient.

Provided, That it meets the tests and methods of assay in § 448.510e(b).

(3) Bacitracin-polymyxin B sulfate topical aerosol containing, in each gram, 500 units of bacitracin and 5,000 units of polymyxin B and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient in a suitable vehicle, packaged in a pressurized container with suitable inert gases: *Provided,* That it meets the tests and methods of assay in § 448.510f(b) of this chapter.

(4) Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ointment containing, in each gram, in a suitable ointment base the following:

(i) 400 units of bacitracin, 3 milligrams of neomycin, 8,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, 5,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient; or

(iii) 500 units of bacitracin, 3.5 milligrams of neomycin, 5,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient; or

(iv) 500 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredieint;

Provided, That it meets the tests and methods of assay in § 448.513c(b) of this chapter.

(5) Bacitracin zinc-polymyxin B sulfate ointment containing, in each gram, 500 units of bacitracin, 10,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient in a suitable ointment base: *Provided,* That it meets the tests and methods of assay in § 448.513a(b) of this chapter.

(6) Neomycin sulfate-polymyxin B sulfate cream containing, in each gram, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and any single generally recognized as safe and effective amine or ''caine''-type local anesthetic active ingredient in a suitable vehicle: *Provided,* That it meets the tests and methods of assay in § 444.542l(b) of this chapter.

[52 FR 47322, Dec. 11, 1987; 52 FR 48792, Dec. 24, 1987, as amended at 53 FR 18838, May 25, 1988; 55 FR 9722, Mar. 15, 1990; 55 FR 40381, Oct. 3, 1990; 55 FR 50172, Dec. 5, 1990]

§ 333.150 Labeling of first aid antibiotic drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''first aid antibiotic.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''First aid to help'' [select one of the following: ''prevent,'' (''decrease'' (''the risk of'' or ''the chance of'')), (''reduce'' (''the risk of'' or ''the chance of'')), ''guard against,'' or ''protect against''] [select one of the following: ''infection,'' ''bacterial contamination,'' or ''skin infection''] ''in minor cuts, scrapes, and burns.'' Other truthful and nonmisleading statements describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''For external use only. Do not use in the eyes or apply over large areas of the body. In case of deep or puncture wounds, animal bites, or serious burns, consult a doctor.''

(2) *For products containing chlortetracycline hydrochloride or tetracycline hydrochloride.*''Stop use and consult a doctor if the condition persists or gets worse. Do not use longer than 1 week unless directed by doctor.''

(3) *For any product containing bacitracin, bacitracin zinc, neomycin, neomycin sulfate, polymyxin B, and/or polymyxin B sulfate.* ''Stop use and consult a doctor if the condition persists or gets worse, or if a rash or other allergic reaction develops. Do not use if you are allergic to any of the ingredients. Do not use longer than 1 week unless directed by a doctor.''

(d) *Directions.* The labeling of the product contains the following statements under the heading ''Directions'': (1) *For ointment and cream products.* ''Clean the affected area. Apply a small amount of this product (an amount equal to the surface area of the tip of a finger) on the area 1 to 3 times daily. May be covered with a sterile bandage.''

(2) *For powder products.* ''Clean the affected area. Apply a light dusting of the powder on the area 1 to 3 times daily. May be covered with a sterile bandage.''

(3) *For aerosol products.* ''Clean the affected area. Spray a small amount of this product on the area 1 to 3 times daily. May be covered with a sterile bandage.''

(e) The word ''doctor'' may be substituted for the word ''physician'' in any of the labeling statements in this subpart.

[52 FR 47332, Dec. 11, 1987, as amended at 61 FR 58472, Nov. 15, 1996]

§ 333.160 Labeling of permitted combinations of active ingredients.

Statements of identity, indications, warnings, and directions for use, respectively, applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable.

(a) *Statement of identity.* For a combination drug product that has an established name, the labeling of the product states the established name of the combination drug product, followed by the statement of identity for each ingredient in the combination, as established in the statement of identity sections of the applicable OTC drug monographs. For a combination drug product that does not have an established name, the labeling of the product states the statement of identity for each ingredient in the combination, as established in the statement of identity sections of the applicable OTC drug monographs.

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the indication(s) for each ingredient in the combination, as established in the ''Indications'' sections of the applicable OTC drug monographs, unless otherwise stated in this paragraph. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For permitted combinations identified in § 333.120(a).* The indications in § 333.150 should be used.

(2) *For permitted combinations identified in § 333.120(b).* In addition to the required indication identified in § 333.150, the labeling of the product may state, under the heading ''Indications,'' the following additional indication: ''First aid for the temporary relief of'' (select one of the following: ''pain,'' ''discomfort,'' ''pain or discomfort'' or ''pain and itching'') ''in minor cuts, scrapes, and burns.''

(c) *Warnings.* The labeling of the product states, under the heading ''Warnings,'' the warning(s) for each ingredient in the combination, as established in the warnings sections of the applicable OTC drug monographs.

(d) *Directions.* The labeling of the product states, under the heading ''Directions,'' directions that conform to the directions established for each ingredient in the directions sections of the applicable OTC drug monographs. When the time intervals or age limitations for administrations of the individual ingredients differ, the directions for the combination product may not exceed any maximum dosage limits established for the individual ingredients in the applicable OTC drug monograph.

Subpart C—Topical Antifungal Drug Products

SOURCE: 58 FR 49898, Sept. 23, 1993, unless otherwise noted.

§ 333.201 Scope.

(a) An over-the-counter antifungal drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart and each general condition established in § 330.1 of this chapter.

(b) Reference in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 333.203 Definitions.

As used in this subpart:

(a) *Antifungal.* A drug which inhibits the growth and reproduction of fungal cells and decreases the number of fungi present.

(b) *Athlete's foot.* An infection of the feet caused by certain dermatophytic fungi.

(c) *Dermatophyte.* A fungus that invades and lives upon the skin or in the hair or nails.

(d) *Fungus.* Any of a large division of plants, including dermatophytes, yeasts, and molds, characterized by a simple cell structure and the absence of chlorophyll.

(e) *Jock itch.* A chronic and recurrent infection caused by certain dermatophytic fungi; affects the upper, inner thighs and sometimes extends to the groin and the pubic area; the condition most frequently occurs in men, but may also occur in women.

(f) *Ringworm.* A skin infection caused by certain dermatophytic fungi.

§ 333.210 Antifungal active ingredients.

The active ingredient of the product consists of any one of the following within the specified concentration established for each ingredient:

(a) Clioquinol 3 percent.

(b) Haloprogin 1 percent.

(c) Miconazole nitrate 2 percent.

(d) Povidone-iodine 10 percent.

(e) Tolnaftate 1 percent.

(f) Undecylenic acid, calcium undecylenate, copper undecylenate, and zinc undecylenate may be used individually or in any ratio that provides a total undecylenate concentration of 10 to 25 percent.

§ 333.250 Labeling of antifungal drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antifungal.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1)(i) of this section and may contain the additional phrase listed in paragraph (b)(1)(ii) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For products containing any ingredient identified in § 333.210 labeled for the treatment of athlete's foot, jock itch, and ringworm.* (i) (Select one of the following: ''Treats,'' ''For the treatment of,'' ''For effective treatment of,'' ''Cures,'' ''For the cure of,'' ''Clears up,'' or ''Proven clinically effective in the treatment of'') (select one condition from any one or more of the following groups of conditions:

(A) ''Athlete's foot,'' athlete's foot (dermatophytosis),'' ''athlete's foot (tinea pedis),'' or ''tinea pedis (athlete's foot)'';

(B) ''Jock itch,'' ''jock itch (tinea cruris),'' or ''tinea cruris (jock itch)''; or

(C) ''Ringworm,'' ''ringworm (tinea corporis),'' or ''tinea corporis (ringworm).'')

(ii) In addition to the information identified in paragraph (b)(1)(i) of this section, the labeling of the product may contain the following statement: (Select one of the following: ''Relieves,'' ''For relief of,'' ''For effective relief of,'' or ''Soothes,'') (select one or more of the following: ''Itching,'' ''scaling,'' ''cracking,'' ''burning,'' ''redness,'' ''soreness,'' ''irritation,'' ''discomfort,'' ''chafing associated with jock itch,'' ''itchy, scaly skin between the toes,'' or ''itching, burning feet'').

(2) *For products containing the ingredient identified in § 333.210(e) labeled for the prevention of athlete's foot.* (i) (Select one of the following: ''Clinically proven to prevent,'' ''Prevents,'' ''Proven effective in the prevention of,'' ''Helps prevent'', ''For the prevention of,'' ''For the prophylaxis (prevention) of,'' ''Guards against,'' or ''Prevents the recurrence of'') (select one of the following: ''Athlete's foot,'' ''athlete's foot (dermatophytosis),'' ''athlete's foot

(tinea pedis),'' or ''tinea pedis (athlete's foot)'') ''with daily use.''

(ii) In addition to the information identified in paragraph (b)(2)(i) of this section, the labeling of the product may contain the following statement: ''Clears up athlete's foot infection and with daily use helps keep it from coming back.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in § 330.210.* (i) ''Do not use on children under 2 years of age unless directed by a doctor.''

(ii) ''For external use only.''

(iii) ''Avoid contact with the eyes.''

(2) *For products labeled according to paragraph (b)(1) of this section for the treatment of athlete's foot and ringworm.* ''If irritation occurs or if there is no improvement within 4 weeks, discontinue use and consult a doctor.''

(3) *For products labeled according to paragraph (b)(1) of this section for the treatment of jock itch.* ''If irritation occurs or if there is no improvement within 2 weeks, discontinue use and consult a doctor.''

(4) *For products labeled according to paragraph (b)(2) of this section for the prevention of athlete's foot.* ''If irritation occurs, discontinue use and consult a doctor.''

(5) *For products containing the ingredient identified in § 333.210(a) labeled according to paragraph (b)(1) of this section.* The following statements must appear in boldface type as the first warnings under the ''Warnings'' heading. (i) ''Do not use on children under 2 years of age.'' (This warning is to be used in place of the warning in paragraph (c)(1)(i) of this section.)

(ii) ''Do not use for diaper rash.''

(d) *Directions.* The labeling of the product contains the following statements under the heading ''Directions'':

(1) *For products labeled according to paragraph (b)(1) of this section for the treatment of athlete's foot, jock itch, and ringworm.* [Select one of the following: ''Clean'' or ''Wash''] ''the affected area and dry thoroughly. Apply'' (the word ''spray'' may be used to replace the word ''apply'' for aerosol products) ''a thin layer of the product over affected area twice daily (morning and night) or as directed by a doctor. Supervise children in the use of this product. For athlete's foot: Pay special attention to spaces between the toes; wear well-fitting, ventilated shoes, and change shoes and socks at least once daily. For athlete's foot and ringworm, use daily for 4 weeks; for jock itch, use daily for 2 weeks. If condition persists longer, consult a doctor. This product is not effective on the scalp or nails.''

(2) *For products labeled according to paragraph (b)(2) of this section for the prevention of athlete's foot.* ''To prevent athlete's foot,'' (select one of the following: ''clean'' or ''wash'') ''the feet and dry thoroughly. Apply'' (the word ''spray'' may be used to replace the word ''apply'' for aerosol products) ''a thin layer of the product to the feet once or twice daily (morning and/or night). Supervise children in the use of this product. Pay special attention to spaces between the toes; wear well-fitting, ventilated shoes, and change shoes and socks at least once daily.''

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

§ 333.280 Professional labeling.

The labeling provided to health professionals (but not to the general public) may contain the following additional indication:

(a) *For products containing haloprogin or miconazole nitrate identified in § 333.210 (a) and (c).* ''For the treatment of superficial skin infections caused by yeast (*Candida albicans*).''

(b) [Reserved]

Subpart D—Topical Acne Drug Products

SOURCE: 56 FR 41019, Aug. 16, 1991, unless otherwise noted.

§ 333.301 Scope.

(a) An over-the-counter acne drug product in a form suitable for topical application is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart and each general condition established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 333.303 Definitions.

As used in this subpart:

(a) *Acne.* A disease involving the oil glands and hair follicles of the skin which is manifested by blackheads, whiteheads, acne pimples, and acne blemishes.

(b) *Acne blemish.* A flaw in the skin resulting from acne.

(c) *Acne drug product.* A drug product used to reduce the number of acne blemishes, acne pimples, blackheads, and whiteheads.

(d) *Acne pimple.* A small, prominent, inflamed elevation of the skin resulting from acne.

(e) *Blackhead.* A condition of the skin that occurs in acne and is characterized by a black tip.

(f) *Whitehead.* A condition of the skin that occurs in acne and is characterized by a small, firm, whitish elevation of the skin.

§ 333.310 Acne active ingredients.

The active ingredient of the product consists of any of the following when labeled according to § 333.350.

(a) Resorcinol 2 percent when combined in accordance with § 333.320(a).

(b) Resorcinol monoacetate 3 percent when combined in accordance with § 333.320(b).

(c) Salicylic acid 0.5 to 2 percent.

(d) Sulfur 3 to 10 percent.

(e) Sulfur 3 to 8 percent when combined in accordance with § 333.320.

§ 333.320 Permitted combinations of active ingredients.

(a) Resorcinol identified in § 333.310(a) when combined with sulfur identified in § 333.310(e) provided the product is labeled according to § 333.350.

(b) Resorcinol monoacetate identified in § 333.310(b) when combined with sulfur identified in § 333.310(e) provided the product is labeled according to § 333.350.

§ 333.350 Labeling of acne drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''acne medication,'' ''acne treatment,'' ''acne medication'' (insert dosage form, *e.g.*, ''cream,'' ''gel,'' ''lotion,'' or ''ointment''), or ''acne treatment'' (insert dosage form, *e.g.*, ''cream,'' ''gel,'' ''lotion,'' or ''ointment'').

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1) of this section and may contain any of the additional phrases listed in paragraph (b)(2) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''For the'' (select one of the following: ''management'' or ''treatment'') ''of acne.''

(2) In addition to the information identified in paragraph (b)(1) of this section, the labeling of the product may contain any one or more of the following statements:

(i) (Select one of the following: ''Clears,'' ''Clears up,'' ''Clears up most,'' ''Dries,'' ''Dries up,'' ''Dries and clears,'' ''Helps clear,'' ''Helps clear up,'' ''Reduces the number of,'' or ''Reduces the severity of'') (select one or more of the following: ''acne blemishes,'' ''acne pimples,'' ''blackheads,'' or ''whiteheads'') which may be followed by ''and allows skin to heal.''

(ii) ''Penetrates pores to'' (select one of the following: ''eliminate most,'' ''control,'' ''clear most,'' or ''reduce the number of'') (select one or more of the following: ''acne blemishes,'' ''acne pimples,'' ''blackheads,'' or ''whiteheads'').

(iii) ''Helps keep skin clear of new'' (select one or more of the following: ''acne blemishes,'' ''acne pimples,'' ''blackheads,'' or ''whiteheads'').

(iv) ''Helps prevent new'' (select one or more of the following: ''acne blemishes,'' ''acne pimples,'' ''blackheads,''

or ''whiteheads'') which may be followed by ''from forming.''

(v) ''Helps prevent the development of new'' (select one or more of the following: ''acne blemishes,'' ''acne pimples,'' ''blackheads,'' or ''whiteheads'').

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in §333.310.* (i) ''For external use only.''

(ii) ''Using other topical acne medications at the same time or immediately following use of this product may increase dryness or irritation of the skin. If this occurs, only one medication should be used unless directed by a doctor.''

(2) *For products containing sulfur identified in §§333.310 (d) and (e).* ''Do not get into eyes. If excessive skin irritation develops or increases, discontinue use and consult a doctor.''

(3) *For products containing any combination identified in §333.320.* ''Apply to affected areas only. Do not use on broken skin or apply to large areas of the body.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) ''Cleanse the skin thoroughly before applying medication. Cover the entire affected area with a thin layer one to three times daily. Because excessive drying of the skin may occur, start with one application daily, then gradually increase to two or three times daily if needed or as directed by a doctor. If bothersome dryness or peeling occurs, reduce application to once a day or every other day.''

(2) The directions described in paragraph (d)(1) of this section are intended for products that are applied and left on the skin. Other products, such as soaps or masks, may be applied and removed and should have appropriate directions.

(3) *Optional directions.* In addition to the required directions in paragraphs (d)(1) and (d)(2) of this section, the product may contain the following optional labeling: ''*Sensitivity Test for a New User.* Apply product sparingly to one or two small affected areas during the first 3 days. If no discomfort occurs, follow the directions stated: (select one of the following: 'elsewhere on this label,' 'above,' or 'below.')''

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

PART 336—ANTIEMETIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
336.1 Scope.
336.3 Definition.

Subpart B—Active Ingredients

336.10 Antiemetic active ingredients.

Subpart C—Labeling

336.50 Labeling of antiemetic drug products.
336.80 Professional labeling.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 52 FR 15892, Apr. 30, 1987, unless otherwise noted.

Subpart A—General Provisions

§336.1 Scope.

(a) An over-the-counter antiemetic drug product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this part and each of the general conditions established in §330.1.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§336.3 Definition.

As used in this part:

Antiemetic. An agent that prevents or treats nausea and vomiting.

Subpart B—Active Ingredients

§336.10 Antiemetic active ingredients.

The active ingredient of the product consists of any of the following when used within the dosage limits established for each ingredient in §336.50(d):

(a) Cyclizine hydrochloride.

(b) Dimenhydrinate.

(c) Diphenhydramine hydrochloride.

(d) Meclizine hydrochloride.

Subpart C—Labeling

§ 336.50 Labeling of antiemetic drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antiemetic.''

(b) *Indications.* The labeling of the product states the following under the heading ''Indications,'' ''For the prevention and treatment of the nausea, vomiting, or dizziness associated with motion sickness.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings:''

(1) *For products containing any ingredient identified in § 336.10*—(i) *When labeled for use in adults and for those products that can be and are labeled for use in children under 12 years of age.* ''Do not take this product, unless directed by a doctor, if you have a breathing problem such as emphysema or chronic bronchitis, or if you have glaucoma or difficulty in urination due to enlargement of the prostate gland.''

(ii) *For those products that can be and are labeled only for children under 12 years of age.* ''Do not give this product to children who have a breathing problem such as chronic bronchitis or who have glaucoma, without first consulting the child's doctor.''

(2) *For products containing cyclizine hydrochloride identified in § 336.10(a).* ''Do not give to children under 6 years of age unless directed by a doctor.''

(3) *For products containing dimenhydrinate identified in § 336.10(b).* ''Do not give to children under 2 years of age unless directed by a doctor.''

(4) *For products containing diphenhydramine hydrochloride identified in § 336.10(c).* ''Do not give to children under 6 years of age unless directed by a doctor.''

(5) *For products containing meclizine hydrochloride identified in § 336.10(d).* ''Do not give to children under 12 years of age unless directed by a doctor.''

(6) *For products containing cyclizine hydrochloride identified in § 336.10(a) or meclizine hydrochloride identified in § 330.10(d).* ''May cause drowsiness; alcohol, sedatives, and tranquilizers may increase the drowsiness effect. Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor. Use caution when driving a motor vehicle or operating machinery.''

(7) *For products containing dimenhydrinate identified in § 336.10(b) or diphenhydramine hydrochloride identified in § 336.10(c).* ''May cause marked drowsiness; alcohol, sedatives, and tranquilizers may increase the drowsiness effect. Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor. Use caution when driving a motor vehicle or operating machinery.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing cyclizine hydrochloride identified in § 336.10(a).* Adults and children 12 years of age and over: Oral dosage is 50 milligrams every 4 to 6 hours, not to exceed 200 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: Oral dosage is 25 milligrams every 6 to 8 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor.

(2) *For products containing dimenhydrinate identified in § 336.10(b).* Adults and children 12 years of age and over: Oral dosage is 50 to 100 milligrams every 4 to 6 hours, not to exceed 400 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12

years of age: Oral dosage is 25 to 50 milligrams every 6 to 8 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Children 2 to under 6 years of age: Oral dosage is 12.5 to 25 milligrams every 6 to 8 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor.

(3) *For products containing diphenhydramine hydrochloride identified in § 336.10(c).* Adults and children 12 years of age and over: Oral dosage is 25 to 50 milligrams every 4 to 6 hours, not to exceed 300 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: Oral dosage is 12.5 to 25 milligrams every 4 to 6 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor.

(4) *For products containing meclizine hydrochloride identified in § 336.10(d).* Adults and children 12 years of age and over: Oral dosage is 25 to 50 milligrams once daily, or as directed by a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[52 FR 15892, Apr. 30, 1987, as amended at 53 FR 35809, Sept. 15, 1988; 59 FR 16982, Apr. 11, 1994]

§ 336.80 Professional labeling.

The labeling provided to health professionals (but not to the general public) may contain the following additional indications.

(a) *For products containing cyclizine hydrochloride, dimenhydrinate, and diphenhydramine hydrochloride identified in § 336.10 (a), (b), and (c).* ''For the treatment of vertigo of motion sickness.''

(b) *For products containing meclizine hydrochloride identified in § 336.10(d).* ''For the treatment of vertigo.''

PART 338—NIGHTTIME SLEEP-AID DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
338.1 Scope.
338.3 Definition.

Subpart B—Active Ingredients

338.10 Nighttime sleep-aid active ingredients.

Subpart C—Labeling

338.50 Labeling of nighttime sleep-aid drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 54 FR 6826, Feb. 14, 1989, unless otherwise noted.

Subpart A—General Provisions

§ 338.1 Scope.

(a) An over-the-counter nighttime sleep-aid drug product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 338.3 Definition.

As used in this part:

Nighttime sleep-aid. A drug that is useful for the relief of occasional sleeplessness by individuals who have difficulty falling asleep.

Subpart B—Active Ingredients

§ 338.10 Nighttime sleep-aid active ingredients.

The active ingredient of the product consists of any of the following when used within the dosage limits established for each ingredient in § 338.50(d):

(a) Diphenhydramine hydrochloride.

(b) Diphenhydramine citrate.

Subpart C—Labeling

§ 338.50 Labeling of nighttime sleep-aid drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''nighttime sleep-aid.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' one or more of the phrases listed in this paragraph. Other truthful and nonmisleading statements, describing only the indications for use

that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) (''Helps you'' or ''Reduces time to'') ''fall asleep if you have difficulty falling asleep.''

(2) ''For relief of occasional sleeplessness.''

(3) ''Helps to reduce difficulty falling asleep.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''Do not give to children under 12 years of age.''

(2) ''If sleeplessness persists continuously for more than 2 weeks, consult your doctor. Insomnia may be a symptom of serious underlying medical illness.''

(3) ''Do not take this product, unless directed by a doctor, if you have a breathing problem such as emphysema or chronic bronchitis, or if you have glaucoma or difficulty in urination due to enlargement of the prostate gland.''

(4) ''Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing diphenhydramine hydrochloride identified in § 338.10(a).* Adults and children 12 years of age and over: Oral dosage is 50 milligrams at bedtime if needed, or as directed by a doctor.

(2) *For products containing diphenhydramine citrate identified in § 338.10(b).* Adults and children 12 years of age and over: Oral dosage is 76 milligrams at bedtime if needed, or as directed by a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[54 FR 6826, Feb. 14, 1989, as amended at 59 FR 16983, Apr. 11, 1994]

PART 340—STIMULANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
340.1 Scope.
340.3 Definition.

Subpart B—Active Ingredient

340.10 Stimulant active ingredient.

Subpart C—Labeling

340.50 Labeling of stimulant drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 53 FR 6105, Feb. 29, 1988, unless otherwise noted.

Subpart A—General Provisions

§ 340.1 Scope.

(a) An over-the-counter stimulant drug product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this part and each of the general conditions established in § 330.1.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 340.3 Definition.

As used in this part:

Stimulant. A drug which helps restore mental alertness or wakefulness during fatigue or drowsiness.

Subpart B—Active Ingredient

§ 340.10 Stimulant active ingredient.

The active ingredient of the product consists of caffeine when used within the dosage limits established in § 340.50(d).

Subpart C—Labeling

§ 340.50 Labeling of stimulant drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''altertness aid'' or a ''stimulant.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''Helps restore mental alertness or wakefulness when experiencing fatigue or drowsiness.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the Act relating to misbranding and the prohibition in section 301(d) of the Act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the Act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''The recommended dose of this product contains about as much caffeine as a cup of coffee. Limit the use of caffeine-containing medications, foods, or beverages while taking this product because too much caffeine may cause nervousness, irritability, sleeplessness, and, occasionally, rapid heart beat.''

(2) ''For occasional use only. Not intended for use as a substitute for sleep. If fatigue or drowsiness persists or continues to recur, consult a'' (select one of the following: ''physician'' or ''doctor'').

(3) ''Do not give to children under 12 years of age.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Adults and children 12 years of age and over: Oral dosage is 100 to 200 milligrams not more often than every 3 to 4 hours.

PART 341—COLD, COUGH, ALLERGY, BRONCHODILATOR, AND ANTIASTHMATIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
341.1 Scope.
341.3 Definitions.

Subpart B—Active Ingredients

341.12 Antihistimine active ingredients.
341.14 Antitussive active ingredients.
341.16 Bronchodilator active ingredients.
341.18 Expectorant active ingredient.
341.20 Nasal decongestant active ingredients.

Subpart C—Labeling

341.70 Labeling of OTC drug products containing ingredients that are used for treating concurrent symptoms (in either a single-ingredient or combination drug product).
341.72 Labeling of antihistimine drug products.
341.74 Labeling of antitussive drug products.
341.76 Labeling of bronchodilator drug products.
341.78 Labeling of expectorant drug products.
341.80 Labeling of nasal decongestant drug products.
341.90 Professional labeling.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

Subpart A—General Provisions

§ 341.1 Scope.

(a) An over-the-counter cold, cough, allergy, bronchodilator, or antiasthmatic drug product in a form suitable for oral, inhalant, or topical administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this part and each of the general conditions established in § 330.1.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

[51 FR 35339, Oct. 2, 1986]

§ 341.3 Definitions.

As used in this part:

(a) *Bronchodilator drug.* A drug used to overcome spasms that cause narrowing of the bronchial air tubes, such as in the symptomatic treatment of the wheezing and shortness of breath of asthma.

(b) *Oral antitussive drug.* A drug that either is taken by mouth or is dissolved in the mouth in the form of a lozenge and acts systemically to relieve cough.

(c) *Topical antitussive drug.* A drug that relieves cough when inhaled after being applied topically to the throat or chest in the form of an ointment or from a steam vaporizer, or when dissolved in the mouth in the form of a lozenge for a local effect.

(d) *Expectorant drug.* A drug taken orally to promote or facilitate the removal of secretions from the respiratory airways.

(e) *Antihistamine drug.* A drug used for the relief of the symptoms of hay fever and upper respiratory allergies (allergic rhinitis).

(f) *Oral nasal decongestant drug.* A drug that is taken by mouth and acts systemically to reduce nasal congestion caused by acute or chronic rhinitis.

(g) *Topical nasal decongestant drug.* A drug that when applied topically inside the nose, in the form of drops, jellies, or sprays, or when inhaled intranasally reduces nasal congestion caused by acute or chronic rhinitis.

(h) *Calibrated dropper.* A dropper calibrated such that the volume error incurred in measuring any liquid does not exceed 15 percent under normal use conditions.

[51 FR 35339, Oct. 2, 1986, as amended at 54 FR 8509, Feb. 28, 1989; 55 FR 40382, Oct. 3, 1990; 57 FR 58374, Dec. 9, 1992; 59 FR 43409, Aug. 23, 1994]

Subpart B—Active Ingredients

§ 341.12 Antihistamine active ingredients.

The active ingredient of the product consists of any of the following when used within the dosage limits established for each ingredient:

(a) Brompheniramine maleate.
(b) Chlorcyclizine hydrochloride.
(c) Chlorpheniramine maleate.
(d) Dexbrompheniramine maleate.
(e) Dexchlorpheniramine maleate.
(f) Diphenhydramine citrate.
(g) Diphenhydramine hydrochloride.
(h) Doxylamine succinate.
(i) Phenindamine tartrate.
(j) Pheniramine maleate.
(k) Pyrilamine maleate.
(l) Thonzylamine hydrochloride.
(m) Triprolidine hydrochloride.

[57 FR 58374, Dec. 9, 1992, as amended at 59 FR 4218, Jan. 28, 1994]

§ 341.14 Antitussive active ingredients.

The active ingredients of the product consist of any of the following when used within the dosage limits and in the dosage forms established for each ingredient in § 341.74(d):

(a) *Oral antitussives.* (1) Chlophedianol hydrochloride.

(2) *Codeine ingredients.* The following ingredients may be used only in combination in accordance with §§ 329.20(a) and 341.40 and 21 CFR 1308.15(c).

(i) Codeine.
(ii) Codeine phosphate.
(iii) Codeine sulfate.
(3) Dextromethorphan.
(4) Dextromethorphan hydrobromide.
(5) Diphenhydramine citrate.
(6) Diphenhydramine hydrochloride.
(b) *Topical antitussives.*
(1) Camphor.
(2) Menthol.

[52 FR 30055, Aug. 12, 1987, as amended at 59 FR 29174, June 3, 1994]

§ 341.16 Bronchodilator active ingredients.

The active ingredients of the product consist of any of the following when used within the dosage limits established for each ingredient:

(a) Ephedrine.
(b) Ephedrine hydrochloride.
(c) Ephedrine sulfate.
(d) Epinephrine.
(e) Epinephrine bitartrate.
(f) Racephedrine hydrochloride.
(g) Racepinephrine hydrochloride.

[51 FR 35339, Oct. 2, 1986]

§ 341.18 Expectorant active ingredient.

The active ingredient of the product is guaifenesin when used within the dosage limits established in § 341.78(d).

[54 FR 8509, Feb. 28, 1989]

§ 341.20 Nasal decongestant active ingredients.

The active ingredient of the product consists of any of the following when used within the dosage limits and in the dosage forms established for each ingredient:

(a) *Oral nasal decongestants.* (1) Phenylephrine hydrochloride.

(2) Pseudoephedrine hydrochloride.

(3) Pseudoephedrine sulfate.

(b) *Topical nasal decongestants.* (1) [Reserved]

(2) Ephedrine.

(3) Ephedrine hydrochloride.

(4) Ephedrine sulfate.

(5) [Reserved]

(6) Naphazoline hydrochloride.

(7) Oxymetazoline hydrochloride.

(8) Phenylephrine hydrochloride.

(9) Propylhexedrine.

(10) Xylometazoline hydrochloride.

[59 FR 43409, Aug. 23, 1994]

Subpart C—Labeling

§ 341.70 Labeling of OTC drug products containing ingredients that are used for treating concurrent symptoms (in either a single-ingredient or combination drug product).

The statements of identity, indications, warnings, and directions for use, respectively, applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable.

(a) *For products containing diphenhydramine citrate and diphenhydramine hydrochloride identified in § 341.14(a)(5) and (a)(6).* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antihistamine/cough suppressant'' or ''antihistamine/antitussive (cough suppressant).'' The indications shall be combined from §§ 341.72(b) and 341.74(b). The warnings shall be combined from §§ 341.72(c)(1), (c)(2), (c)(4), and (c)(6) and 341.74(c)(1), (c)(2), (c)(3), and (c)(4). Alternatively, all of the warnings in § 341.74(c) shall be used. The directions for OTC labeling shall follow §§ 341.74(d)(1)(iv) or (d)(1)(v), as applicable. The directions for professional labeling shall follow § 341.90(j) or (k), as applicable.

(b) (Reserved)

[61 FR 15703, Apr. 9, 1996]

§ 341.72 Labeling of antihistamine drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''antihistamine.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in paragraph (b) of this section, as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''Temporarily'' (select one of the following: ''relieves,'' ''alleviates,'' ''decreases,'' ''reduces,'' or ''dries'') ''runny nose and'' (select one of the following: ''relieves,'' ''alleviates,'' ''decreases,'' or ''reduces'') ''sneezing, itching of the nose or throat, and itchy, watery eyes due to hay fever'' (which may be followed by one or both of the following: ''or other upper respiratory allergies'' or ''(allergic rhinitis)'').

(2) ''For the temporary relief of runny nose, sneezing, itching of the nose or throat, and itchy, watery eyes due to hay fever'' (which may be followed by one or both of the following: ''or other upper respiratory allergies'' or ''(allergic rhinitis)'').

(c) *Warnings.* The labeling of the product contains the following warnings, under the heading ''Warnings'':

(1) ''May cause excitability especially in children.''

(2) ''Do not take this product, unless directed by a doctor, if you have a

breathing problem such as emphysema or chronic bronchitis, or if you have glaucoma or difficulty in urination due to enlargement of the prostate gland.''

(3) *For products containing brompheniramine maleate, chlorcyclizine hydrochloride, chlorpheniramine maleate, dexbrompheniramine maleate, dexchlorpheniramine maleate, phenindamine tartrate, pheniramine maleate, pyrilamine maleate, thonzylamine hydrochloride, or triprolidine hydrochloride identified in §341.12(a), (b), (c), (d), (e), (i), (j), (k), (l), and (m).* ''May cause drowsiness; alcohol, sedatives, and tranquilizers may increase the drowsiness effect. Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor. Use caution when driving a motor vehicle or operating machinery.''

(4) *For products containing diphenhydramine citrate, diphenhydramine hydrochloride, or doxylamine succinate identified in §341.12(f), (g), and (h).* ''May cause marked drowsiness; alcohol, sedatives, and tranquilizers may increase the drowsiness effect. Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor. Use caution when driving a motor vehicle or operating machinery.''

(5) *For products containing phenindamine tartrate identified in §341.12(i).* ''May cause nervousness and insomnia in some individuals.''

(6) *For products that are labeled only for use by children under 12 years of age.* The labeling of the product contains only the warnings identified in paragraphs (c)(1) and (c)(5) of this section as well as the following:

(i) ''Do not give this product to children who have a breathing problem such as chronic bronchitis, or who have glaucoma, without first consulting the child's doctor.''

(ii) *For products containing brompheniramine maleate, chlorpheniramine maleate, dexbrompheniramine maleate, dexchlorpheniramine maleate, phenindamine tartrate, pheniramine maleate, pyrilamine maleate, thonzylamine hydrochloride, or triprolidine hydrochloride identified in §341.12(a), (c), (d), (e), (i), (j), (k), (l), and (m).* ''May cause drowsiness. Sedatives and tranquilizers may increase the drowsiness effect. Do not give this product to children who are taking sedatives or tranquilizers, without first consulting the child's doctor.''

(iii) *For products containing diphenhydramine citrate, diphenhydramine hydrochloride, or doxylamine succinate identified in §341.12(f), (g), and (h).* ''May cause marked drowsiness. Sedatives and tranquilizers may increase the drowsiness effect. Do not give this product to children who are taking sedatives or tranquilizers, without first consulting the child's doctor.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing brompheniramine maleate identified in §341.12(a).* Adults and children 12 years of age and over: oral dosage is 4 milligrams every 4 to 6 hours, not to exceed 24 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 2 milligrams every 4 to 6 hours, not to exceed 12 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(2) *For products containing chlorcyclizine hydrochloride identified in §341.12(b).* Adults and children 12 years of age and over: oral dosage is 25 milligrams every 6 to 8 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor. Children under 12 years of age: consult a doctor.

(3) *For products containing chlorpheniramine maleate identified in §341.12(c).* Adults and children 12 years of age and over: oral dosage is 4 milligrams every 4 to 6 hours, not to exceed 24 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 2 milligrams every 4 to 6 hours, not to exceed 12 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(4) *For products containing dexbrompheniramine maleate identified in §341.12(d).* Adults and children 12 years

of age and over: oral dosage is 2 milligrams every 4 to 6 hours, not to exceed 12 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 1 milligram every 4 to 6 hours, not to exceed 6 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(5) *For products containing dexchlorpheniramine maleate identified in § 341.12(e).* Adults and children 12 years of age and over: oral dosage is 2 milligrams every 4 to 6 hours, not to exceed 12 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 1 milligram every 4 to 6 hours, not to exceed 6 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(6) *For products containing diphenhydramine citrate identified in § 341.12(f).* Adults and children 12 years of age and over: oral dosage is 38 to 76 milligrams every 4 to 6 hours, not to exceed 456 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 19 to 38 milligrams every 4 to 6 hours, not to exceed 228 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(7) *For products containing diphenhydramine hydrochloride identified in § 341.12(g).* Adults and children 12 years of age and over: oral dosage is 25 to 50 milligrams every 4 to 6 hours, not to exceed 300 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 12.5 to 25 milligrams every 4 to 6 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(8) *For products containing doxylamine succinate identified in § 341.12(h).* Adults and children 12 years of age and over: oral dosage is 7.5 to 12.5 milligrams every 4 to 6 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 3.75 to 6.25 milligrams every 4 to 6 hours, not to exceed 37.5 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(9) *For products containing phenindamine tartrate identified in § 341.12(i).* Adults and children 12 years of age and over: oral dosage is 25 milligrams every 4 to 6 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 12.5 milligrams every 4 to 6 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(10) *For products containing pheniramine maleate identified in § 341.12(j).* Adults and children 12 years of age and over: oral dosage is 12.5 to 25 milligrams every 4 to 6 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 6.25 to 12.5 milligrams every 4 to 6 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(11) *For products containing pyrilamine maleate identified in § 341.12(k).* Adults and children 12 years of age and over: oral dosage is 25 to 50 milligrams every 6 to 8 hours, not to exceed 200 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 12.5 to 25 milligrams every 6 to 8 hours, not to exceed 100 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(12) *For products containing thonzylamine hydrochloride identified in § 341.12(l).* Adults and children 12 years of age and over: oral dosage is 50 to 100 milligrams every 4 to 6 hours, not to exceed 600 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 25 to 50 milligrams every 4 to 6 hours, not to exceed 300 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(13) *For products containing triprolidine hydrochloride identified in § 341.12(m).* Adults and children 12 years of age and over: oral dosage is 2.5 milligrams every 4 to 6 hours, not to exceed 10 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 1.25 milligrams every 4 to 6 hours, not to exceed 5 milligrams in 24 hours, or as directed by a

doctor. Children under 6 years of age: consult a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[57 FR 58374, Dec. 9, 1992, as amended at 59 FR 4218, Jan. 28, 1994]

§ 341.74 Labeling of antitussive drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''cough suppressant'' or an ''antitussive (cough suppressant).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in this paragraph (b), as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph, may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''Temporarily'' (select one of the following: ''alleviates,'' ''calms,'' ''controls,'' ''decreases,'' ''quiets,'' ''reduces,'' ''relieves,'' or ''suppresses'') ''cough due to'' (select one of the following: ''minor bronchial irritation'' or ''minor throat and bronchial irritation'') (select one of the following: ''as may occur with,'' ''associated with,'' or ''occurring with'') (select one of the following: ''A cold'' or ''the common cold'') ''or inhaled irritants.''

(2) ''Temporarily'' (select one of the following: ''alleviates,'' ''calms,'' ''controls,'' ''decreases,'' ''quiets,'' ''reduces,'' ''relieves,'' or ''suppresses'') ''cough'' (select one of the following: ''as may occur with,'' ''associated with,'' or ''occurring with'') (select one of the following: ''A cold,'' ''the common cold,'' or ''inhaled irritants'').

(3) In addition to the required information identified in paragraphs (b) (1) and (2) of this section, the labeling of the product may contain any (one or more) of the following statements:

(i) ''Cough suppressant which temporarily'' (select one of the following: ''Alleviates,'' ''controls,'' ''decreases,'' ''reduces,'' ''relieves,'' or ''suppresses'') ''the impulse to cough.''

(ii) ''Temporarily helps you cough less.''

(iii) ''Temporarily helps to'' (select one of the following: ''Alleviate,'' ''control,'' ''decrease,'' ''reduce,'' ''relieve,'' or ''suppress'') ''the cough reflex that causes coughing.''

(iv) ''Temporarily'' (select one of the following: ''Alleviates,'' ''controls,'' ''decreases,'' ''reduces,'' ''relieves,'' or ''suppresses'') ''the intensity of coughing.''

(v) (Select one of the following: ''Alleviates,'' ''Controls,'' ''Decreases,'' ''Reduces,'' ''Relieves,'' or ''Suppresses'') (select one of the following: ''Cough,'' ''the impulse to cough,'' or ''your cough'') ''to help you'' (select one of the following: ''Get to sleep,'' ''sleep,'' or ''rest'').

(vi) *For products containing chlophedianol hydrochloride, codeine ingredients, dextromethorphan, or dextromethorphan hydrobromide identified in § 341.14(a) (1), (2), (3), and (4).* ''Calms the cough control center and relieves coughing.''

(vii) *For products containing chlophedianol hydrochloride, dextromethorphan, dextromethorphan hydrobromide, camphor, or menthol identified in § 341.14(a) (1), (3), (4) and (b) (1) and (2).* (*a*) ''Nonnarcotic cough suppressant for the temporary'' (select one of the following: ''alleviation,'' ''control,'' ''decrease,'' ''reduction,'' ''relief,'' or ''suppression'') ''of cough.''

(*b*) (Select one of the following: ''Alleviates,'' ''Controls,'' ''Decreases,'' ''Reduces,'' ''Relieves,'' or ''Suppresses'') ''cough impulses without narcotics.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For oral and topical antitussives.* ''A persistent cough may be a sign of a serious condition. If cough persists for more than 1 week, tends to recur, or is accompanied by fever, rash, or persistent headache, consult a doctor.''

(2) *For oral and topical antitussives labeled for adults or for adults and children under 12 years of age.* ''Do not take this

product for persistent or chronic cough such as occurs with smoking, asthma, or emphysema, or if cough is accompanied by excessive phlegm (mucus) unless directed by a doctor.''

(3) *For oral and topical antitussives labeled only for children under 12 years of age.* ''Do not give this product for persistent or chronic cough such as occurs with asthma or if cough is accompanied by excessive phlegm (mucus) unless directed by a doctor.''

(4) *Oral antitussives*—(i) *For products containing codeine ingredients identified in §341.14(a)(2).* ''May cause or aggravate constipation.''

(ii) *For products containing codeine ingredients identified in §341.14(a)(2) when labeled only for adults.* ''Do not take this product if you have a chronic pulmonary disease or shortness of breath unless directed by a doctor.''

(iii) *For products containing codeine ingredients identified in §341.14(a)(2) when labeled only for children under 12 years of age.* ''Do not give this product to children who have a chronic pulmonary disease, shortness of breath, or who are taking other drugs unless directed by a doctor.''

(iv) *For products containing codeine ingredients identified in §341.14(a)(2) when labeled for use in adults and children under 12 years of age.* ''Adults and children who have a chronic pulmonary disease or shortness of breath, or children who are taking other drugs, should not take this product unless directed by a doctor.''

(v) *For products containing dextromethorphan or dextromethorphan hydrobromide as identified in §341.14(a)(3) and (a)(4) when labeled for adults or for adults and children under 12 years of age.* ''*Drug interaction precaution.* Do not use this product if you are now taking a prescription monoamine oxidase inhibitor (MAOI) (certain drugs for depression, psychiatric or emotional conditions, or Parkinson's disease), or for 2 weeks after stopping the MAOI drug. If you are uncertain whether your prescription drug contains an MAOI, consult a health professional before taking this product.''

(vi) *For products containing dextromethorphan or dextromethorphan hydrobromide as identified in §341.14(a)(3) and (a)(4) when labeled only for children under 12 years of age.* ''*Drug interaction precaution.* Do not give this product to a child who is taking a prescription monoamine oxidase inhibitor (MAOI) (certain drugs for depression, psychiatric or emotional conditions), or for 2 weeks after stopping the MAOI drug. If you are uncertain whether your child's prescription drug contains an MAOI, consult a health professional before giving this product.''

(vii) *For products containing diphenhydramine citrate or diphenhydramine hydrochloride identified in §341.14(a)(5) and (a)(6).* ''May cause excitability especially in children.''

(viii) *For products containing diphenhydramine citrate or diphenhydramine hydrochloride identified in §341.14(a)(5) and (a)(6) when labeled only for children under 12 years of age*—(A) ''Do not give this product to children who have a breathing problem such as chronic bronchitis, or who have glaucoma, without first consulting the child's doctor.''

(B) ''May cause marked drowsiness. Sedatives and tranquilizers may increase the drowsiness effect. Do not give this product to children who are taking sedatives or tranquilizers, without first consulting the child's doctor.''

(ix) *For products containing diphenhydramine citrate or diphenhydramine hydrochloride identified in §341.14(a)(5) and (a)(6) when labeled for use in adults and children under 12 years of age*—(A) ''Do not take this product, unless directed by a doctor, if you have a breathing problem such as emphysema or chronic bronchitis, or if you have glaucoma or difficulty in urination due to enlargement of the prostate gland.''

(B) ''May cause marked drowsiness; alcohol, sedatives, and tranquilizers may increase the drowsiness effect. Avoid alcoholic beverages while taking this product. Do not take this product if you are taking sedatives or tranquilizers, without first consulting your doctor. Use caution when driving a motor vehicle or operating machinery.''

(5) *Topical antitussives*—(i) *For products containing camphor or menthol identified in § 341.14(b) (1) and (2) in a suitable ointment vehicle.* ''For external use only. Do not take by mouth or place in nostrils.''

(ii) *For products containing camphor or menthol identified in § 341.14(b) (1) and (2) for steam inhalation use.* ''For steam inhalation only. Do not take by mouth.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *Oral antitussives*—(i) *For products containing chlophedianol hydrochloride identified in § 341.14(a)(1).* Adults and children 12 years of age and over: Oral dosage is 25 milligrams every 6 to 8 hours, not to exceed 100 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: Oral dosage is 12.5 milligrams every 6 to 8 hours, not to exceed 50 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: Consult a doctor.

(ii) *For products containing codeine ingredients identified in § 341.14(a)(2).* Adults and children 12 years of age and over: Oral dosage is 10 to 20 milligrams every 4 to 6 hours, not to exceed 120 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: Oral dosage is 5 to 10 milligrams every 4 to 6 hours, not to exceed 60 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: Consult a doctor. A special measuring device should be used to give an accurate dose of this product to children under 6 years of age. Giving a higher dose than recommended by a doctor could result in serious side effects for your child.

(iii) *For products containing dextromethorphan or dextromethorphan hydrobromide identified in § 341.14(a) (3) and (4).* The dosage is equivalent to dextromethorphan hydrobromide. Adults and children 12 years of age and over: Oral dosage is 10 to 20 milligrams every 4 hours or 30 milligrams every 6 to 8 hours, not to exceed 120 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: Oral dosage is 5 to 10 milligrams every 4 hours or 15 milligrams every 6 to 8 hours, not to exceed 60 milligrams in 24 hours, or as directed by a doctor. Children 2 to under 6 years of age: Oral dosage is 2.5 to 5 milligrams every 4 hours or 7.5 milligrams every 6 to 8 hours, not to exceed 30 milligrams in 24 hours, or as directed by a doctor. Children under 2 years of age: Consult a doctor.

(iv) *For products containing diphenhydramine citrate identified in § 341.14(a)(5).* Adults and children 12 years of age and over: oral dosage is 38 milligrams every 4 hours, not to exceed 228 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 19 milligrams every 4 hours, not to exceed 114 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(v) *For products containing diphenhydramine hydrochloride identified in § 341.14(a)(6).* Adults and children 12 years of age and over: oral dosage is 25 milligrams every 4 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Children 6 to under 12 years of age: oral dosage is 12.5 milligrams every 4 hours, not to exceed 75 milligrams in 24 hours, or as directed by a doctor. Children under 6 years of age: consult a doctor.

(2) *Topical antitussives*—(i) *For products containing camphor identified in § 341.14(b)(1) in a suitable ointment vehicle.* The product contains 4.7 to 5.3 percent camphor. Adults and children 2 to under 12 years of age: Rub on the throat and chest as a thick layer. The area of application may be covered with a warm, dry cloth if desired. However, clothing should be left loose about the throat and chest to help the vapors rise to reach the nose and mouth. Applications may be repeated up to three times daily or as directed by a doctor. Children under 2 years of age: consult a doctor.

(ii) *For products containing menthol identified in § 341.14(b)(2) in a suitable ointment vehicle.* The product contains 2.6 to 2.8 percent menthol. Adults and children 2 to under 12 years of age: Rub on the throat and chest as a thick layer. The area of application may be covered with a warm, dry cloth if desired. However, clothing should be left loose about the throat and chest to help the vapors rise to reach the nose

and mouth. Applications may be repeated up to three times daily or as directed by a doctor. Children under 2 years of age: consult a doctor.

(iii) *For products containing menthol identified in § 341.14(b)(2) in a lozenge.* The product contains 5 to 10 milligrams menthol. Adults and children 2 to under 12 years of age: Allow lozenge to dissolve slowly in the mouth. May be repeated every hour as needed or as directed by a doctor. Children under 2 years of age: Consult a doctor.

(iv) *For products containing camphor identified in § 341.14(b)(1) for steam inhalation use.* The product contains 6.2 percent camphor. Adults and children 2 to under 12 years of age: Add 1 tablespoonful of solution, for each quart of water, directly to the water in a hot steam vaporizer, bowl, or wash basin; or add 1½ teaspoonsful of solution, for each pint of water, to an open container of boiling water. Breathe in the medicated vapors. May be repeated up to three times daily or as directed by a doctor. Children under 2 years of age: consult a doctor.

(v) *For products containing menthol identified in § 341.14(b)(2) for steam inhalation use.* The product contains 3.2 percent menthol. Adults and children 2 to under 12 years of age: Add 1 tablespoonful of solution, for each quart of water, directly to the water in a hot steam vaporizer, bowl, or wash basin; or add 1½ teaspoonsful of solution, for each pint of water, to an open container of boiling water. Breathe in the medicated vapors. May be repeated up to three times daily or as directed by a doctor. Children under 2 years of age: consult a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

(f) *Exemption from the general accidental overdose warning.* The labeling for antitussive drug products containing the active ingredient identified in § 341.14(b)(2) marketed in accordance with § 341.74(d)(2)(iii) is exempt from the requirement in § 330.1(g) of this chapter that the labeling bear the general warning statement ''In case of accidental overdose, seek professional assistance or contact a poison control center immediately.'' The labeling must continue to bear the first part of the general warning in § 330.1(g) of this chapter, which states, ''Keep this and all drugs out of the reach of children.''

[52 FR 30055, Aug. 12, 1987; 52 FR 35610, Sept. 22, 1987; 53 FR 35809, Sept. 15, 1988; 55 FR 27808, July 6, 1990; 55 FR 40383, Oct. 3, 1990; 58 FR 54236, Oct. 20, 1993; 59 FR 29174, June 3, 1994; 59 FR 36051, July 15, 1994]

§ 341.76 Labeling of bronchodilator drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''bronchodilator.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''For temporary relief of shortness of breath, tightness of chest, and wheezing due to bronchial asthma.''

(2) In addition to the required information identified in paragraph (b)(1) of this section, the labeling of the product may contain one or more of the following statements:

(i) ''For the'' (select one of the following: ''temporary relief'' or ''symptomatic control'') ''of bronchial asthma.''

(ii) ''Eases breathing for asthma patients'' (which may be followed by: ''by reducing spasms of bronchial muscles'').

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''Do not use this product unless a diagnosis of asthma has been made by a doctor.''

(2) ''Do not use this product if you have heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland unless directed by a doctor.''

(3) ''Do not use this product if you have ever been hospitalized for asthma or if you are taking any prescription drug for asthma unless directed by a doctor.''

(4) ''*Drug interaction precaution*. Do not use this product if you are now taking a prescription monoamine oxidase inhibitor (MAOI) (certain drugs for depression, psychiatric or emotional conditions, or Parkinson's disease), or for 2 weeks after stopping the MAOI drug. If you are uncertain whether your prescription drug contains an MAOI, consult a health professional before taking this product.''

(5) *For products containing ephedrine, ephedrine hydrochloride, ephedrine sulfate, or racephedrine hydrochloride identified in § 341.16 (a), (b), (c), and (f).* (i) ''Do not continue to use this product, but seek medical assistance immediately if symptoms are not relieved within 1 hour or become worse.''

(ii) ''Some users of this product may experience nervousness, tremor, sleeplessness, nausea, and loss of appetite. If these symptoms persist or become worse, consult your doctor.''

(6) *For products containing epinephrine, epinephrine bitartrate, or racepinephrine hydrochloride identified in § 341.16 (d), (e), and (g).* (i) ''Do not use this product more frequently or at higher doses than recommended unless directed by a doctor. [first sentence in boldface type] Excessive use may cause nervousness and rapid heart beat, and, possibly, adverse effects on the heart.''

(ii) ''Do not continue to use this product, but seek medical assistance immediately if symptoms are not relieved within 20 minutes or become worse.'' [sentence in boldface type]

(iii) *For products intended for use in a hand-held rubber bulb nebulizer.* ''Do not use this product if it is brown in color or cloudy.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing ephedrine, ephedrine hydrochloride, ephedrine sulfate, or racephedrine hydrochloride identified in § 341.16 (a), (b), (c), and (f).* Adults and children 12 years of age and over: Oral dosage is 12.5 to 25 milligrams every 4 hours, not to exceed 150 milligrams in 24 hours, or as directed by a doctor. Do not exceed recommended dose unless directed by a doctor. Children under 12 years of age: Consult a doctor.

(2) *For products containing epinephrine, epinephrine bitartrate, and racepinephrine hydrochloride identified in § 341.16(d), (e), and (g) for use in a hand-held rubber bulb nebulizer.* The ingredient is used in an aqueous solution at a concentration equivalent to 1 percent epinephrine. Inhalation dosage for adults, children 12 years of age and over, and children 4 to under 12 years of age: 1 to 3 inhalations not more often than every 3 hours. The use of this product by children should be supervised by an adult. Children under 4 years of age: Consult a doctor.

(Collection of information requirement approved by the Office of Management and Budget under control number 0910–0237)

[51 FR 35339, Oct. 2, 1986, as amended at 52 FR 7126, Mar. 9, 1987; 52 FR 7830, Mar. 13, 1987; 53 FR 35810, Sept. 15, 1988; 58 FR 54242, Oct. 20, 1993; 61 FR 25146, May 20, 1996; 62 FR 9684, Mar. 4, 1997]

§ 341.78 Labeling of expectorant drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''expectorant.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''Helps loosen phlegm (mucus) and thin bronchial secretions to'' (select one or more of the following: ''rid the bronchial passageways of bothersome mucus,'' ''drain bronchial tubes,'' and ''make coughs more productive''). Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings, under the heading ''Warnings'':

(1) ''A persistent cough may be a sign of a serious condition. If cough persists for more than 1 week, tends to recur, or is accompanied by a fever, rash, or persistent headache, consult a doctor.''

(2) *For expectorant drug products labeled for adults or for adults and children under 12 years of age.* ''Do not take this product for persistent or chronic cough such as occurs with smoking, asthma, chronic bronchitis, or emphysema, or where cough is accompanied by excessive phlegm (mucus) unless directed by a doctor.''

(3) *For expectorant drug products labeled only for children under 12 years of age.* ''Do not give this product for persistent or chronic cough such as occurs with asthma or if cough is accompanied by excessive phlegm (mucus) unless directed by a doctor.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'' for products containing guaifenesin identified in § 341.18: Adults and children 12 years of age and over: oral dosage is 200 to 400 milligrams every 4 hours not to exceed 2,400 milligrams in 24 hours. Children 6 to under 12 years of age: oral dosage is 100 to 200 milligrams every 4 hours not to exceed 1,200 milligrams in 24 hours. Children 2 to under 6 years of age: oral dosage is 50 to 100 milligrams every 4 hours not to exceed 600 milligrams in 24 hours. Children under 2 years of age: consult a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[54 FR 8509, Feb. 28, 1989, as amended at 57 FR 29177, June 30, 1992]

§ 341.80 Labeling of nasal decongestant drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''nasal decongestant.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1) of this section, as appropriate, and may contain any additional phrases listed in paragraph (b)(2) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraphs (b)(1) and (b)(2) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) (Select one of the following: ''For the temporary relief of nasal congestion'' or ''Temporarily relieves nasal congestion'') (which may be followed by any of the following in paragraphs (b)(1) (i), (ii), and (iii) of this section):

(i) ''due to'' (select one of the following: ''the common cold'' or ''a cold'').

(ii) ''due to'' (select one of the following: ''hay fever,'' ''hay fever (allergic rhinitis),'' ''hay fever or other upper respiratory allergies,'' or ''hay fever or other upper respiratory allergies (allergic rhinitis)'').

(iii) ''associated with sinusitis.''

(2) In addition to the information identified in paragraph (b)(1) of this section, the labeling of the product may contain any (one or more) of the following statements:

(i) (Select one of the following: ''For the temporary relief of'' or ''Temporarily relieves'') (select one of the following: ''stuffy nose,'' ''stopped up nose,'' ''nasal stuffiness,'' or ''clogged up nose.'')

(ii) (Select one of the following: ''Reduces swelling of,'' ''Decongests,'' or ''Helps clear'') ''nasal passages; shrinks swollen membranes.''

(iii) ''Temporarily restores freer breathing through the nose.''

(iv) ''Helps decongest sinus openings and passages; temporarily relieves sinus congestion and pressure.''

(v) ''Promotes nasal and/or sinus drainage; temporarily relieves sinus congestion and pressure.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *Oral nasal decongestants*—(i) *For products containing phenylephrine hydrochloride, pseudoephedrine hydrochloride, or pseudoephedrine sulfate identified in § 341.20 (a)(1), (a)(2), and (a)(3) when labeled for adults.* (A) ''Do not exceed

recommended dosage. [first sentence in boldface type] If nervousness, dizziness, or sleeplessness occur, discontinue use and consult a doctor.''

(B) ''If symptoms do not improve within 7 days or are accompanied by fever, consult a doctor.''

(C) ''Do not take this product if you have heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland unless directed by a doctor.''

(D) ''*Drug interaction precaution.* Do not use this product if you are now taking a prescription monoamine oxidase inhibitor (MAOI) (certain drugs for depression, psychiatric or emotional conditions, or Parkinson's disease), or for 2 weeks after stopping the MAOI drug. If you are uncertain whether your prescription drug contains an MAOI, consult a health professional before taking this product.''

(ii) *For products containing phenylephrine hydrochloride, pseudoephedrine hydrochloride, or pseudoephedrine sulfate identified in § 341.20 (a)(1), (a)(2), and (a)(3) when labeled for children under 12 years of age.* (A) ''Do not exceed recommended dosage. [first sentence in boldface type] If nervousness, dizziness, or sleeplessness occur, discontinue use and consult a doctor.''

(B) ''If symptoms do not improve within 7 days or are accompanied by fever, consult a doctor.''

(C) ''Do not give this product to a child who has heart disease, high blood pressure, thyroid disease, or diabetes unless directed by a doctor.''

(D) ''*Drug interaction precaution.* Do not give this product to a child who is taking a prescription monoamine oxidase inhibitor (MAOI) (certain drugs for depression, psychiatric or emotional conditions), or for 2 weeks after stopping the MAOI drug. If you are uncertain whether your child's prescription drug contains an MAOI, consult a health professional before giving this product.''

(iii) *For oral nasal decongestant products labeled for both adults and children under 12 years of age.* The labeling of the product contains the warnings identified in paragraph (c)(1)(i) of this section.

(2) *Topical nasal decongestants*—(i) *For products containing any topical nasal decongestant identified in § 341.20(b) when labeled for adults.* (A) ''Do not exceed recommended dosage.'' [sentence in boldface type]

(B) ''This product may cause temporary discomfort such as burning, stinging, sneezing, or an increase in nasal discharge.''

(C) ''The use of this container by more than one person may spread infection.''

(ii) [Reserved]

(iii) *For products containing ephedrine, ephedrine hydrochloride, ephedrine sulfate, naphazoline hydrochloride, oxymetazoline hydrochloride, phenylephrine hydrochloride, or xylometazoline hydrochloride identified in § 341.20 (b)(2), (b)(3), (b)(4), (b)(6), (b)(7), (b)(8), and (b)(10) when used as nasal sprays, drops, or jellies and when labeled for adults.* (A) ''Do not use this product for more than 3 days. Use only as directed. Frequent or prolonged use may cause nasal congestion to recur or worsen. If symptoms persist, consult a doctor.''

(B) ''Do not use this product if you have heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland unless directed by a doctor.''

(iv) *For products containing naphazoline hydrochloride identified in § 341.20(b)(6) at a concentration of 0.05 percent.* ''Do not use this product in children under 12 years of age because it may cause sedation if swallowed.''

(v) *For products containing propylhexedrine identified in § 341.20(b)(9) when used in an inhalant dosage form and when labeled for adults.* ''Do not use this product for more than 3 days. Use only as directed. Frequent or prolonged use may cause nasal congestion to recur or worsen. If symptoms persist, consult a doctor.''

(vi) *For products containing any topical nasal decongestant identified in § 341.20(b) when labeled for children under 12 years of age.* The labeling of the product contains the warnings identified in paragraph (c)(2)(i) of this section.

(vii) [Reserved]

(viii) *For products containing ephedrine, ephedrine hydrochloride, ephedrine sulfate, naphazoline hydrochloride,*

oxymetazoline hydrochloride, phenylephrine hydrochloride, or xylometazoline hydrochloride identified in § 341.20(b)(2), (b)(3), (b)(4), (b)(6), (b)(7), (b)(8), and (b)(10) when used as nasal sprays, drops, or jellies and when labeled for children under 12 years of age. (A) ''Do not use this product for more than 3 days. Use only as directed. Frequent or prolonged use may cause nasal congestion to recur or worsen. If symptoms persist, consult a doctor.''

(B) ''Do not use this product in a child who has heart disease, high blood pressure, thyroid disease, or diabetes unless directed by a doctor.''

(ix) *For products containing propylhexedrine identified in § 341.20(b)(9) when used in an inhalant dosage form and when labeled for children under 12 years of age.* ''Do not use this product for more than 3 days. Use only as directed. Frequent or prolonged use may cause nasal congestion to recur or worsen. If symptoms persist, consult a doctor.''

(x) *For topical nasal decongestant products labeled for both adults and for children under 12 years of age.* The labeling of the product contains the applicable warnings identified in paragraphs (c)(2)(i), (c)(2)(ii), (c)(2)(iii), and (c)(2)(v) of this section.

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *Oral nasal decongestants*—(i) *For products containing phenylephrine hydrochloride identified in § 341.20(a)(1).* Adults and children 12 years of age and over: 10 milligrams every 4 hours not to exceed 60 milligrams in 24 hours. Children 6 to under 12 years of age: 5 milligrams every 4 hours not to exceed 30 milligrams in 24 hours. Children 2 to under 6 years of age: 2.5 milligrams every 4 hours not to exceed 15 milligrams in 24 hours. Children under 2 years of age: consult a doctor.

(ii) *For products containing pseudoephedrine hydrochloride or pseudoephedrine sulfate identified in § 341.20 (a)(2) and (a)(3).* Adults and children 12 years of age and over: 60 milligrams every 4 to 6 hours not to exceed 240 milligrams in 24 hours. Children 6 to under 12 years of age: 30 milligrams every 4 to 6 hours not to exceed 120 milligrams in 24 hours. Children 2 to under 6 years of age: 15 milligrams every 4 to 6 hours not to exceed 60 milligrams in 24 hours. Children under 2 years of age: consult a doctor.

(2) *Topical nasal decongestants*—(i) [Reserved]

(ii) *For products containing ephedrine, ephedrine hydrochloride, or ephedrine sulfate identified in § 341.20(b) (2), (3), and (4)*—(A) *Nasal drops or sprays—For a 0.5-percent aqueous solution.* Adults and children 12 years of age and over: 2 or 3 drops or sprays in each nostril not more often than every 4 hours. Children 6 to under 12 years of age (with adult supervision): 1 or 2 drops or sprays in each nostril not more often than every 4 hours. Children under 6 years of age: consult a doctor.

(B) *Nasal jelly—For a 0.5-percent water-based jelly.* Adults and children 6 to under 12 years of age (with adult supervision): place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 4 hours.

(iii) *For products containing naphazoline hydrochloride identified in § 341.20(b)(6)*—(A) *Nasal drops or sprays—(1) For a 0.05-percent aqueous solution.* Adults and children 12 years of age and over: 1 or 2 drops or sprays in each nostril not more often than every 6 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *For a 0.025-percent aqueous solution.* Children 6 to under 12 years of age (with adult supervision): 1 or 2 drops or sprays in each nostril not more often than every 6 hours. Children under 6 years of age: consult a doctor.

(B) *Nasal jelly—(1) For a 0.05-percent water-based jelly.* Adults and children 12 years of age and over: place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 6 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *For a 0.025-percent water-based jelly.* Children 6 to under 12 years of age (with adult supervision): place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 6 hours. Children under 6 years of age: consult a doctor.

(iv) *For products containing oxymetazoline hydrochloride identified in § 341.20(b)(7)*—(A) *Nasal drops or sprays*—(*1*) *For a 0.05-percent aqueous solution.* Adults and children 6 to under 12 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 10 to 12 hours. Do not exceed 2 doses in any 24-hour period. Children under 6 years of age: consult a doctor.

(*2*) *A 0.025-percent aqueous solution in a container having either a calibrated dropper or a metered-dose spray that delivers no more than 0.027 milligrams of oxymetazoline per three drops or three sprays.* Children 2 to under 6 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 10 to 12 hours. Use only recommended amount. Do not exceed 2 doses in any 24-hour period. [previous two sentences in boldface type] Children under 2 years of age: consult a doctor.

(B) *Nasal jelly—For a 0.05-percent water-based jelly.* Adults and children 6 to under 12 years of age (with adult supervision): place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 10 to 12 hours. Do not exceed 2 doses in any 24-hour period. Children under 6 years of age: consult a doctor.

(v) *For products containing phenylephrine hydrochloride identified in § 341.20(b)(8)*—(A) *Nasal drops or sprays*—(*1*) *For a 1-percent aqueous solution.* Adults and children 12 years of age and over: 2 or 3 drops or sprays in each nostril not more often than every 4 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *For a 0.5-percent aqueous solution.* Adults and children 12 years of age and over: 2 or 3 drops or sprays in each nostril not more often than every 4 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*3*) *For a 0.25-percent aqueous solution.* Adults and children 6 to under 12 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 4 hours. Children under 6 years of age: consult a doctor.

(*4*) *A 0.125-percent aqueous solution in a container having either a calibrated dropper or a metered-dose spray that delivers no more than 0.135 milligrams of phenylephrine per three drops or three sprays.* Children 2 to under 6 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 4 hours. Use only recommended amount. [previous sentence in boldface type] Children under 2 years of age: consult a doctor.

(B) *Nasal jelly*—(*1*) *For a 1-percent water-based jelly.* Adults and children 12 years of age and over: place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 4 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *For a 0.5-percent water-based jelly.* Adults and children 12 years of age and over: place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 4 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*3*) *For a 0.25-percent water-based jelly.* Adults and children 6 to under 12 years of age (with adult supervision): place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 4 hours. Children under 6 years of age: consult a doctor.

(vi) *For products containing propylhexedrine identified in § 341.20(b)(9) when used in an inhalant dosage form.* The product delivers in each 800 milliliters of air 0.40 to 0.50 milligrams of propylhexedrine. Adults and children 6 to under 12 years of age (with adult supervision): 2 inhalations in each nostril not more often than every 2 hours. Children under 6 years of age: consult a doctor.

(vii) *For products containing xylometazoline hydrochloride identified in § 341.20(b)(10)*—(A) *Nasal drops or sprays*—(*1*) *For a 0.1-percent aqueous solution.* Adults and children 12 years of age and over: 2 or 3 drops or sprays in each nostril not more often than every 8 to 10 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *A 0.05-percent aqueous solution in a container having either a calibrated dropper or a metered-dose spray that delivers no more than 0.054 milligrams of*

xylometazoline per three drops or three sprays. Children 6 to under 12 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 8 to 10 hours. Children 2 to under 6 years of age (with adult supervision): 2 or 3 drops or sprays in each nostril not more often than every 8 to 10 hours. Use only recommended amount. Do not exceed 3 doses in any 24-hour period. [previous two sentences in boldface type] Children under 2 years of age: consult a doctor.

(B) *Nasal jelly—(1) For a 0.1-percent water-based jelly.* Adults and children 12 years of age and over: place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 8 to 10 hours. Do not give to children under 12 years of age unless directed by a doctor.

(*2*) *For a 0.05-percent water-based jelly.* Children 6 to under 12 years of age (with adult supervision): place a small amount in each nostril and inhale well back into the nasal passages. Use not more often than every 8 to 10 hours. Children under 6 years of age: consult a doctor.

(viii) *Other required statements—For products containing propylhexedrine identified in § 341.20(b)(9) when used in an inhalant dosage form.* (A) ''This inhaler is effective for a minimum of 3 months after first use.''

(B) ''Keep inhaler tightly closed.''

[59 FR 43409, Aug. 23, 1994]

§ 341.90 Professional labeling.

The labeling of the product provided to health professionals (but not to the general public) may contain the following additional dosage information for products containing the active ingredients identified below:

(a) *For products containing ephedrine, ephedrine hydrochloride, ephedrine sulfate, or racephedrine hydrochloride identified in § 341.16 (a), (b), (c), and (f).* Children 6 to under 12 years of age: oral dosage is 6.25 to 12.5 milligrams every 4 hours, not to exceed 75 milligrams in 24 hours. Children 2 to under 6 years of age: oral dosage is 0.3 to 0.5 milligram per kilogram of body weight every 4 hours, not to exceed 2 milligrams per kilogram of body weight in 24 hours.

(b) *For products containing chlophedianol hydrochloride identified in 341.14(a)(1).* Children 2 to under 6 years of age: oral dosage is 12.5 milligrams every 6 to 8 hours, not to exceed 50 milligrams in 24 hours.

(c) *For products containing codeine ingredients identified in § 341.14(a)(2).* (1) Children 2 to under 6 years of age: Oral dosage is 1 milligram per kilogram body weight per day administered in four equal divided doses. The average body weight for each age may also be used to determine dosage as follows: For children 2 years of age (average body weight, 12 kilograms), the oral dosage is 3 milligrams every 4 to 6 hours, not to exceed 12 milligrams in 24 hours; for children 3 years of age (average body weight, 14 kilograms), the oral dosage is 3.5 milligrams every 4 to 6 hours, not to exceed 14 milligrams in 24 hours; for children 4 years of age (average body weight, 16 kilograms), the oral dosage is 4 milligrams every 4 to 6 hours, not to exceed 16 milligrams in 24 hours: for children 5 years of age (average body weight, 18 kilograms), the oral dosage is 4.5 milligrams every 4 to 6 hours, not to exceed 18 milligrams in 24 hours. The manufacturer must relate these dosages for its specific product dosages for its specific product to the use of the calibrated measuring device discussed in paragraph (c)(3) of this section. If age is used to determine the dose, the directions must include instructions to reduce the dose for low-weight children.

(2) Parents should be instructed to obtain and use a calibrated measuring device for administering the drug to the child, to use extreme care in measuring the dosage, and not exceed the recommended daily dosage.

(3) A dispensing device (such as a dropper calibrated for age or weight) should be dispensed along with the product when it is intended for use in children 2 to under 6 years of age to prevent possible overdose due to improper measuring of the dose.

(4) Codeine is not recommended for use in children under 2 years of age. Children under 2 years may be more susceptible to the respiratory depressant effects of codeine, including respiratory arrest, coma, and death.

(d) *The following labeling indication may be used for products containing guaifenesin identified in § 341.18 when used as a single ingredient product.* ''Helps loosen phlegm and thin bronchial secretions in patients with stable chronic bronchitis.''

(e) *For products containing brompheniramine maleate identified in § 341.12(a).* Children 2 to under 6 years of age: oral dosage is 1 milligram every 4 to 6 hours, not to exceed 6 milligrams in 24 hours.

(f) *For products containing chlorcyclizine hydrochloride identified in § 341.12(b).* Children 6 to under 12 years of age: oral dosage is 12.5 milligrams every 6 to 8 hours, not to exceed 37.5 milligrams in 24 hours. Children 2 to under 6 years of age: oral dosage is 6.25 milligrams every 6 to 8 hours, not to exceed 18.75 milligrams in 24 hours.

(g) *For products containing chlorpheniramine maleate identified in § 341.12(c).* Children 2 to under 6 years of age: oral dosage is 1 milligram every 4 to 6 hours, not to exceed 6 milligrams in 24 hours.

(h) *For products containing dexbrompheniramine maleate identified in § 341.12(d).* Children 2 to under 6 years of age: oral dosage is 0.5 milligram every 4 to 6 hours, not to exceed 3 milligrams in 24 hours.

(i) *For products containing dexchlorpheniramine maleate identified in § 341.12(e).* Children 2 to under 6 years: oral dosage is 0.5 milligram every 4 to 6 hours, not to exceed 3 milligrams in 24 hours.

(j) *For products containing diphenhydramine citrate identified in § 341.12(f).* Children 2 to under 6 years of age: oral dosage is 9.5 milligrams every 4 to 6 hours, not to exceed 57 milligrams in 24 hours.

(k) *For products containing diphenhydramine hydrochloride identified in § 341.12(g).* Children 2 to under 6 years of age: oral dosage is 6.25 milligrams every 4 to 6 hours, not to exceed 37.5 mg in 24 hours.

(l) *For products containing doxylamine succinate identified in § 341.12(h).* Children 2 to under 6 years of age: oral dosage is 1.9 to 3.125 milligrams every 4 to 6 hours, not to exceed 18.75 milligrams in 24 hours.

(m) *For products containing phenindamine tartrate identified in § 341.12(i).* Children 2 to under 6 years of age: oral dosage is 6.25 milligrams every 4 to 6 hours, not to exceed 37.5 milligrams in 24 hours.

(n) *For products containing pheniramine maleate identified in § 341.12(j).* Children 2 to under 6 years of age: oral dosage is 3.125 to 6.25 milligrams every 4 to 6 hours, not to exceed 37.5 milligrams in 24 hours.

(o) *For products containing pyrilamine maleate identified in § 341.12(k).* Children 2 to under 6 years of age: oral dosage is 6.25 to 12.5 milligrams every 6 to 8 hours, not to exceed 50 milligrams in 24 hours.

(p) *For products containing thonzylamine hydrochloride identified in § 341.12(l).* Children 2 to under 6 years of age: oral dosage is 12.5 to 25 milligrams every 4 to 6 hours, not to exceed 150 milligrams in 24 hours.

(q) *For products containing triprolidine hydrochloride identified in § 341.12(m).* Children 4 to under 6 years of age: oral dosage is 0.938 milligram every 4 to 6 hours, not to exceed 3.744 milligrams in 24 hours. Children 2 to under 4 years of age: oral dosage is 0.625 milligram every 4 to 6 hours, not to exceed 2.5 milligrams in 24 hours. Infants 4 months to under 2 years of age: oral dosage is 0.313 milligram every 4 to 6 hours, not to exceed 1.252 milligrams in 24 hours.

(r) *For products containing diphenhydramine citrate identified in § 341.14(a)(5).* Children 2 to under 6 years of age: oral dosage is 9.5 milligrams every 4 hours, not to exceed 57 milligrams in 24 hours.

(s) *For products containing diphenhydramine hydrochloride identified in § 341.14(a)(6).* Children 2 to under 6 years of age: oral dosage is 6.25 milligrams every 4 hours, not to exceed 37.5 milligrams in 24 hours.

[51 FR 35339, Oct. 2, 1986, as amended at 52 FR 30057, Aug. 12, 1987; 54 FR 8509, Feb. 28, 1989; 57 FR 58376, Dec. 9, 1992; 59 FR 4218, Jan. 28, 1994; 59 FR 29174, June 3, 1994; 59 FR 36051, July 15, 1994]

PART 344—TOPICAL OTIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
344.1 Scope.
344.3 Definitions.

Subpart B—Active Ingredients

344.10 Topical otic active ingredient.

Subpart C—Labeling

344.50 Labeling of topical otic drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 51 FR 28660, Aug. 8, 1986, unless otherwise noted:

Subpart A—General Provisions

§ 344.1 Scope.

(a) An over-the-counter topical otic drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this part in addition to each of the general conditions established in § 330.1.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 344.3 Definitions.

As used in this part:

(a) *Anhydrous glycerin.* An ingredient that may be prepared by heating glycerin U.S.P. at 150° C for 2 hours to drive off the moisture content.

(b) *Earwax removal aid.* A drug used in the external ear canal that aids in the removal of excessive earwax.

Subpart B—Active Ingredients

§ 344.10 Topical otic active ingredient.

The active ingredient of the product consists of carbamide peroxide 6.5 percent formulated in an anhydrous glycerin vehicle.

Subpart C—Labeling

§ 344.50 Labeling of topical otic drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''earwax removal aid.''

(b) *Indication.* The labeling of the product states, under the heading ''Indication,'' the following: ''For occasional use as an aid to'' (which may be followed by: ''soften, loosen, and'') ''remove excessive earwax.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''Do not use if you have ear drainage or discharge, ear pain, irritation, or rash in the ear or are dizzy; consult a doctor.''

(2) ''Do not use if you have an injury or perforation (hole) of the ear drum or after ear surgery unless directed by a doctor.''

(3) ''Do not use for more than 4 days; if excessive earwax remains after use of this product, consult a doctor.''

(4) ''Avoid contact with the eyes.''

(d) *Directions.* The labeling of the product contains the following statement under the heading ''Directions'': FOR USE IN THE EAR ONLY. Adults and children over 12 years of age: tilt head sideways and place 5 to 10 drops into ear. Tip of applicator should not enter ear canal. Keep drops in ear for several minutes by keeping head tilted or placing cotton in the ear. Use twice daily for up to 4 days if needed, or as directed by a doctor. Any wax remaining after treatment may be removed by gently flushing the ear with warm water, using a soft rubber bulb ear syringe. Children under 12 years of age: consult a doctor.

(e) *Optional wording.* The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[51 FR 28660, Aug. 8, 1986; 52 FR 7830, Mar. 13, 1987]

PART 346—ANORECTAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
346.1 Scope.
346.3 Definitions.

Subpart B—Active Ingredients

346.10 Local anesthetic active ingredients.
346.12 Vasoconstrictor active ingredients.
346.14 Protectant active ingredients.
346.16 Analgesic, anesthetic, and antipruritic active ingredients.
346.18 Astringent active ingredients.
346.20 Keratolytic active ingredients.
346.22 Permitted combinations of anorectal active ingredients.

Subpart C—Labeling

346.50 Labeling of anorectal drug products.
346.52 Labeling of permitted combinations of anorectal active ingredients.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 55 FR 31779, Aug. 3, 1990, unless otherwise noted.

Subpart A—General Provisions

§ 346.1 Scope.

(a) An over-the-counter anorectal drug product in a form suitable for external (topical) or intrarectal (rectal) administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 212 unless otherwise noted.

§ 346.3 Definitions.

As used in this part:

(a) *Analgesic, anesthetic drug.* A topically (externally) applied drug that relieves pain by depressing cutaneous sensory receptors.

(b) *Anorectal drug.* A drug that is used to relieve symptoms caused by anorectal disorders in the anal canal, perianal area, and/or the lower rectal areas.

(c) *Antipruritic drug.* A topically (externally) applied drug that relieves itching by depressing cutaneous sensory receptors.

(d) *Astringent drug.* A drug that is applied topically (externally) to the skin or mucous membranes for a local and limited protein coagulant effect.

(e) *External use.* Topical application of an anorectal drug product to the skin of the perianal area and/or the skin of the anal canal.

(f) *Intrarectal use.* Topical application of an anorectal drug product to the mucous membrane of the rectum.

(g) *Keratolytic drug.* A drug that causes desquamation (loosening) and debridement or sloughing of the surface cells of the epidermis.

(h) *Local anesthetic drug.* A drug that produces local disappearance of pain, burning, itching, irritation, and/or discomfort by reversibly blocking nerve conduction when applied to nerve tissue in appropriate concentrations.

(i) *Protectant drug.* A drug that provides a physical barrier, forming a protective coating over skin or mucous membranes.

(j) *Vasoconstrictor.* A drug that causes temporary constriction of blood vessels.

Subpart B—Active Ingredients

§ 346.10 Local anesthetic active ingredients.

The active ingredient of the product consists of any of the following when used in the concentration or within the concentration range established for each ingredient:

(a) Benzocaine 5 to 20 percent.

(b) Benzyl alcohol 1 to 4 percent.

(c) Dibucaine 0.25 to 1 percent.

(d) Dibucaine hydrochloride 0.25 to 1 percent.

(e) Dyclonine hydrochloride 0.5 to 1 percent.

(f) Lidocaine 2 to 5 percent.

(g) Pramoxine hydrochloride 1 percent.

(h) Tetracaine 0.5 to 1 percent.

(i) Tetracaine hydrochloride 0.5 to 1 percent.

§ 346.12 Vasoconstrictor active ingredients.

The active ingredient of the product consists of any of the following when used in the concentration or within the concentration range established for each ingredient.

(a) Ephedrine sulfate 0.1 to 1.25 percent.

(b) Epinephrine 0.005 to 0.01 percent.

(c) Epinephrine hydrochloride 0.005 to 0.01 percent.

(d) Phenylephrine hydrochloride 0.25 percent.

§ 346.14 Protectant active ingredients.

(a) The following active ingredients may be used as the sole protectant active ingredient in a product if the ingredient as identified constitutes 50 percent or more by weight of the final product. In addition, the following active ingredients may be used in concentrations of less than 50 percent by weight only when used in combinations in accordance with § 346.22 (a), (b), or (n).

(1) Aluminum hydroxide gel.

(2) Cocoa butter.

(3) Glycerin in a 20- to 45-percent (weight/weight) aqueous solution so that the final product contains not less than 10 and not more than 45 percent glycerin (weight/weight). Any combination product containing glycerin must contain at least this minimum amount of glycerin.

(4) Hard fat.

(5) Kaolin.

(6) Lanolin.

(7) Mineral oil.

(8) Petrolatum.

(9) Topical starch.

(10) White petrolatum.

(b) The following active ingredients may not be used as a sole protectant ingredient but may be used in combination with one, two, or three other protectant active ingredients in accordance with § 346.22 (a), (b), (n), and (o) and with the following limitations:

(1) Calamine not to exceed 25 percent by weight per dosage unit (based on the zinc oxide content of calamine).

(2) Cod liver oil, provided that the product is labeled so that the amount of the product that is used in a 24-hour period represents a quantity that provides 10,000 U.S.P. units of vitamin A and 400 U.S.P. units of cholecalciferol.

(3) Shark liver oil, provided that the product is labeled so that the amount of the product that is used in a 24-hour period represents a quantity that provides 10,000 U.S.P. units of vitamin A and 400 U.S.P. units of cholecalciferol.

(4) Zinc oxide not to exceed 25 percent by weight per dosage unit.

§ 346.16 Analgesic, anesthetic, and antipruritic active ingredients.

The active ingredient of the product consists of any of the following when used within the concentration range established for each ingredient:

(a) Camphor 0.1 to 3 percent.

(b) Juniper tar 1 to 5 percent.

(c) Menthol 0.1 to 1 percent.

§ 346.18 Astringent active ingredients.

The active ingredient of the product consists of any of the following when used within the concentration range established for each ingredient:

(a) Calamine, within a concentration range of 5 to 25 percent by weight per dosage unit (based on the zinc oxide content of calamine).

(b) Witch hazel, 10 to 50 percent.

(c) Zinc oxide, within a concentration range of 5 to 25 percent by weight per dosage unit.

[55 FR 31779, Aug. 3, 1990, as amended at 59 FR 28767, June 3, 1994]

§ 346.20 Keratolytic active ingredients.

The active ingredient of the product consists of any of the following when used within the concentration range established for each ingredient:

(a) Alcloxa 0.2 to 2 percent.

(b) Resorcinol 1 to 3 percent.

§ 346.22 Permitted combinations of anorectal active ingredients.

(a) Any two, three, or four protectants identified in (a) § 346.14 may be combined, except aluminum hydroxide gel in § 346.14(a)(1) and kaolin in § 346.14(a)(5) may not be combined with any ingredient in § 346.14(a) (2), (4), (6), (7), (8) and (10), and (b) (2) and (3), provided that the combined percentage by weight of all protectants in the combination is at least 50 percent of

the final product (*e.g.*, 1 gram of a 2-gram dosage unit). Any protectant ingredient included in the combination must be present at a level that contributes at least 12.5 percent by weight (*e.g.*, 0.25 gram of a 2-gram dosage unit), except cod liver oil and shark liver oil. If an ingredient in §346.14(b) is included in the combination, it must not exceed the concentration limit specified in §346.14(b).

(b) Any single anorectal ingredient identified in §346.10, 346.12, 346.16, 346.18, or 346.20 may be combined with up to four protectants in accordance with paragraph (a) of this section.

(c) Any single local anesthetic identified in §346.10 may be combined with any single vasoconstrictor identified in §346.12.

(d) Any single local anesthetic identified in §346.10 may be combined with any single astringent identified in §346.18.

(e) Any single local anesthetic identified in §346.10 may be combined with any single keratolytic identified in §346.20.

(f) Any single vasoconstrictor identified in §346.12 may be combined with any single astringent identified in §346.18.

(g) Any single analgesic, anesthetic, and antipruritic identified in §346.16 may be combined with any single astringent identified in §346.18.

(h) Any single analgesic, anesthetic, and antipruritic identified in §346.16 may be combined with any single keratolytic identified in §346.20.

(i) Any single astringent identified in §346.18 may be combined with any single keratolytic identified in §346.20.

(j) Any single local anesthetic identified in §346.10 may be combined with any single vasoconstrictor identified in §346.12 and with any single astringent identified in §346.18.

(k) Any single local anesthetic identified in §346.10 may be combined with any single astringent identified in §346.18 and with any single keratolytic identified in §346.20.

(l) Any single vasoconstrictor identified in §346.12 may be combined with any single analgesic, anesthetic, and antipruritic identified in §346.16 and with any single astringent identified in §346.18.

(m) Any single analgesic, anesthetic, and antipruritic identified in §346.16 may be combined with any single astringent identified in §346.18 and with any single keratolytic identified in §346.20.

(n) Any combination of ingredients listed in paragraphs (c) through (m) of this section may be combined with up to four protectants in accordance with paragraph (a) of this section.

(o) Any product containing calamine for use as a protectant and/or as an astringent and/or containing zinc oxide for use as a protectant and/or as an astringent may not have a total weight of zinc oxide exceeding 25 percent by weight per dosage unit.

Subpart C—Labeling

§346.50 Labeling of anorectal drug products.

The labeling of the product contains the following information for anorectal ingredients identified in §§346.10, 346.12, 346.14, 346.16, 346.18, and 346.20, and for combinations of anorectal ingredients identified in §346.22. Unless otherwise specified, the labeling in this subpart is applicable to anorectal drug products for both external and intrarectal use.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as ''anorectal (hemorrhoidal),'' ''hemorrhoidal,'' ''hemorrhoidal (anorectal) (insert dosage form, e.g., cream, lotion, or ointment).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in paragraph (b) of this section, as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph, may also be used, as provided in §330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) (''For the temporary relief of,'' ''Gives temporary relief of,'' or ''Helps

relieve the'') (As an option, select one or both of the following: ''local'' or ''anorectal'') [select one or more of the following: ''discomfort,'' ''itching,'' or ''itching and discomfort,'' followed by: ''in the perianal area'' or ''associated with'' (select one or more of the following: ''hemorrhoids,'' ''anorectal disorders,'' ''inflamed hemorrhoidal tissues,'' ''anorectal inflammation,'' ''hemorrhoidal tissues,'' or ''piles (hemorrhoids).'')]

(2) *Additional indications.* Indications applicable to each active ingredient of the product may be combined to eliminate duplicative words or phrases so that the resulting indication is clear and understandable. In addition to the indication identified in paragraph (b)(1) of this section, the labeling of the product intended for external or intrarectal use may also contain the following indications, as appropriate.

(i) *For products for external use only containing any ingredient identified in § 346.10.* ''For the temporary relief of'' (select one or more of the following: ''pain,'' ''soreness,'' or ''burning'').

(ii) *For products containing epinephrine or epinephrine hydrochloride identified in § 346.12 (b) and (c) for external use only, and for products containing ephedrine sulfate or phenylephrine hydrochloride identified in § 346.12 (a) and (d).*

(A) ''Temporarily reduces the swelling associated with'' (select one of the following: ''irritated hemorrhoidal tissue and other anorectal disorders'' or ''irritation in hemorrhoids and other anorectal disorders'').

(B) ''Temporarily shrinks hemorrhoidal tissue.''

(iii) *For products for external use only containing glycerin identified in § 346.14(a)(3) and for products for external and/or intrarectal use containing any protectant identified in § 346.14(a) (2), (4), (6) through (10), and (b) (1) through (4).*

(A) ''Temporarily forms a protective coating over inflamed tissues to help prevent drying of tissues.''

(B) ''Temporarily protects irritated areas.''

(C) ''Temporarily relieves burning.''

(D) ''Provides temporary relief from skin irritations.''

(E) ''Temporarily provides a coating for relief of anorectal discomforts.''

(F) ''Temporarily protects the inflamed, irritated anorectal surface'' (select one of the following: ''to help make bowel movements less painful'' or ''from irritation and abrasion during bowel movement'').

(G) ''Temporarily protects inflamed perianal skin.''

(H) ''Temporarily relieves the symptoms of perianal skin irritation.''

(iv) *For products containing aluminum hydroxide gel identified in § 346.14(a)(1) and for products containing kaolin identified in § 346.14(a)(5).* ''For the temporary relief of itching associated with moist anorectal conditions.''

(v) *For products for external use only containing any analgesic, anesthetic, and antipruritic identified in § 346.16.*

(A) ''For the temporary relief of'' (select one or both of the following: ''pain'' or ''burning'').

(B) ''Can help distract from pain.''

(C) ''May provide a cooling sensation.''

(vi) *For products for external use only containing witch hazel identified in § 346.18(b), and for products for external use and/or intrarectal use containing calamine or zinc oxide identified in § 346.18 (a) and (c).*

(A) ''Aids in protecting irritated anorectal areas.''

(B) ''Temporary relief of'' (select one or both of the following: ''irritation'' or ''burning'').

(vii) *For products for external use only containing any ingredient identified in § 346.20.* The indication in paragraph (b)(1) of this section applies.

(c) *Warnings.* Warnings applicable to each active ingredient of the product may be combined to eliminate duplicative words or phrases so that the resulting warning is clear and understandable. The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''If condition worsens or does not improve within 7 days, consult a doctor.''

(2) ''Do not exceed the recommended daily dosage unless directed by a doctor.''

(3) ''In case of bleeding, consult a doctor promptly.''

(4) *For products for external use only.* ''Do not put this product into the rectum by using fingers or any mechanical device or applicator.''

(5) *For products for intrarectal use to be used with a special applicator such as a pile pipe or other mechanical device.* ''Do not use this product with an applicator if the introduction of the applicator into the rectum causes additional pain. Consult a doctor promptly.''

(6) *For products for external use only containing any local anesthetic identified in § 346.10, menthol identified in § 346.16(c), or resorcinol identified in § 346.20(b).* ''Certain persons can develop allergic reactions to ingredients in this product. If the symptom being treated does not subside or if redness, irritation, swelling, pain, or other symptoms develop or increase, discontinue use and consult a doctor.''

(7) *For products containing any vasoconstrictor identified in § 346.12.* (i) ''Do not use this product if you have heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland unless directed by a doctor.''

(ii) ''*Drug interaction precaution.* Do not use this product if you are presently taking a prescription drug for high blood pressure or depression, without first consulting your doctor.''

(iii) *For products containing ephedrine sulfate identified in § 346.12(a).* ''Some users of this product may experience nervousness, tremor, sleeplessness, nausea, and loss of appetite. If these symptoms persist or become worse, consult your doctor.''

(8) *For products containing aluminum hydroxide gel identified in § 346.14(a)(1) and for products containing kaolin identified in § 346.14(a)(5).* ''Remove petrolatum or greasy ointment before using this product because they interfere with the ability of this product to adhere properly to the skin area.''

(9) *For products for external use only containinq resorcinol identified in § 346.20(b).* ''Do not use on open wounds near the anus.''

(d) *Directions.* Directions applicable to each active ingredient of the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable. The labeling of the product contains the following information under the heading ''Directions'':

(1) ''*Adults:* When practical, cleanse the affected area'' (select one or both of the following: ''with mild soap and warm water and rinse thoroughly'' or ''by patting or blotting with an appropriate cleansing pad''). ''Gently dry by patting or blotting with toilet tissue or a soft cloth before application of this product.'' [Other appropriate directions in this section may be inserted here.] ''Children under 12 years of age: consult a doctor.''

(2) *For products for external use only.* ''Apply externally to the affected area'' (insert appropriate time interval of administration as identified in paragraphs (d)(6), (7), (8), or (9) of this section).

(3) *For products for external use that are pads containing anorectal ingredients.* ''Gently apply to the affected area by patting and then discard.''

(4) *For products for intrarectal use that are wrapped suppositories.* ''Remove wrapper before inserting into the rectum.''

(5) *For products for intrarectal use that are to be used with a special applicator such as a pile pipe or other mechanical device.* ''FOR INTRARECTAL USE: Attach applicator to tube. Lubricate applicator well, then gently insert applicator into the rectum.''

(6) *For products for external use only containing any of the local anesthetics identified in § 346.10; analgesics, anesthetics, and antipruritics identified in § 346.16; or alcloxa or resorcinol identified in § 346.20.* Apply to the affected area up to 6 times daily.

(i) *For products for external use only containing dibucaine or dibucaine hydrochloride identified in § 346.10 (c) and (d).* Apply to the affected area up to 3 or 4 times daily.

(ii) *For products for external use only containing pramoxine hydrochloride identified in § 346.10(g).* Apply to the affected area up to 5 times daily.

(7) *For products containing vasoconstrictors identified in § 346.12.* Apply to the affected area up to 4 times daily.

(8) *For products for external use only containing glycerin identified in § 346.14(a)(3) or witch hazel identified in § 346.18(b), and for products for external and/or intrarectal use containing any*

protectant identified in § 346.14(a)(1), (2), (4), (5), (6), (7), and (9), and (b)(1), (2), (3), and (4), or any astringent identified in § 346.18(a) and (c). Apply to the affected area up to 6 times daily or after each bowel movement.

(9) *For products containing petrolatum or white petrolatum identified in § 346.14(a)(8) and (10).* Apply liberally to the affected area as often as necessary.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[55 FR 31779, Aug. 3, 1990, as amended at 59 FR 28767, June 3, 1994]

§ 346.52 Labeling of permitted combinations of anorectal active ingredients.

Indications, warnings, and directions for use, respectively, applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable.

(a) *Statement of identity.* For a combination drug product that has an established name, the labeling of the product states the established name of the combination drug product, followed by the statement of identity established in § 346.50(a). For a combination drug product that does not have an established name, the labeling of the product states the statement of identity established in § 346.50(a).

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the indication(s) for each ingredient in the combination, as established in the indications sections of this subpart.

(c) *Warnings.* The labeling of the product states, under the heading ''Warnings,'' the warning(s) for each ingredient in the combination, as established in the warnings sections of this subpart.

(d) *Directions.* The labeling of the product states, under the heading ''Directions,'' directions that conform to the directions established for each ingredient in the directions sections of this subpart. When the time intervals or age limitations for administration of the individual ingredients differ, the directions for the combination product may not exceed any maximum dosage limits established for the individual ingredients in the applicable OTC drug monograph.

PART 347—SKIN PROTECTANT DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—Astringent Drug Products

Sec.
347.1 Scope.
347.3 Definitions.
347.10 Astringent active ingredients.
347.50 Labeling of astringent drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 58 FR 54462, Oct. 21, 1993, unless otherwise noted.

Subpart A—Astringent Drug Products

§ 347.1 Scope.

(a) An over-the-counter skin protectant drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 347.3 Definitions.

As used in this part:

(a) *Astringent drug product* means a drug product that is applied to the skin or mucous membranes for a local and limited protein coagulant effect.

(b) [Reserved]

§ 347.10 Astringent active ingredients.

The active ingredient of the product consists of any one of the following within the specified concentration established for each ingredient:

(a) Aluminum acetate, 0.13 to 0.5 percent (depending on the formulation and concentration of the marketed product, the manufacturer must provide adequate directions so that the resulting solution to be used by the consumer contains 0.13 to 0.5 percent aluminum acetate).

(b) Aluminum sulfate, 46 to 63 percent (the concentration is based on the anhydrous equivalent).

(c) Witch hazel.

[58 FR 54462, Oct. 21, 1993, as amended at 59 FR 28768, June 3, 1994]

§347.50 Labeling of astringent drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''astringent.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications'' any of the phrases listed in this paragraph (b), as appropriate. Other truthful and nonmisleading statements describing only the indications for use that have been established and listed in this paragraph (b) may also be used, as provided in §330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For products containing aluminum acetate identified in §347.10(a).* ''For temporary relief of minor skin irritations due to'' (select one or more of the following: ''poison ivy,'' ''poison oak,'' ''poison sumac,'' ''insect bites,'' ''athlete's foot,'' or ''rashes caused by soaps, detergents, cosmetics, or jewelry'').

(2) *For products containing aluminum sulfate identified in §347.10(b) for use as a styptic pencil.* ''Stops bleeding caused by minor surface cuts and abrasions as may occur during shaving.''

(3) *For products containing witch hazel identified in §347.10(c).* (i) ''For relief of minor skin irritations due to'' (select one or more of the following: ''insect bites,'' ''minor cuts,'' or ''minor scrapes'').

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''For external use only. Avoid contact with the eyes.''

(2) *For products containing aluminum acetate identified in §347.10(a) or witch hazel identified in §347.10(c).* ''If condition worsens or symptoms persist for more than 7 days, discontinue use of the product and consult a'' (select one of the following: ''physician'' or ''doctor'').

(3) *For products containing aluminum acetate identified in §347.10(a) used as a compress or wet dressing.* ''Do not cover compress or wet dressing with plastic to prevent evaporation.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing aluminum acetate identified in §347.10(a)*—(i) *For products used as a soak.* ''For use as a soak: Soak affected area in the solution for 15 to 30 minutes. Discard solution after each use. Repeat 3 times a day.''

(ii) *For products used as a compress or wet dressing.* ''For use as a compress or wet dressing: saturate a clean, soft, white cloth (such as a diaper or torn sheet) in the solution, gently squeeze, and apply loosely to the affected area. Saturate the cloth in the solution every 15 to 30 minutes and apply to the affected area. Discard solution after each use. Repeat as often as necessary.''

(2) *For products containing aluminum sulfate identified in §347.10(b) for use as a styptic pencil.* ''Moisten tip of pencil with water and apply to the affected area. Dry pencil after use.''

(3) *For products containing witch hazel identified in §347.10(c).* ''Apply to the affected area as often as necessary.''

[58 FR 54462, Oct. 21, 1993, as amended at 59 FR 28768, June 3, 1994]

PART 348—EXTERNAL ANALGESIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
348.1 Scope.
348.3 Definitions.

Subpart B—Active Ingredients

348.10 Analgesic, anesthetic, and antipruritic active ingredients.

Subpart C—Labeling

348.50 Labeling of external analgesic drug

products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 57 FR 27656, June 19, 1992, unless otherwise noted.

Subpart A—General Provisions

§ 348.1 Scope.

(a) An over-the-counter external analgesic drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 348.3 Definitions.

As used in this part:

(a) *Male genital desensitizing drug product*. A drug product applied to the penis to help in temporarily slowing the onset of ejaculation.

(b) [Reserved]

Subpart B—Active Ingredients

§ 348.10 Analgesic, anesthetic, and antipruritic active ingredients.

The active ingredient of the product consists of any of the following within the specified concentration established for each ingredient:

(a) *Male genital desensitizers*. (1) Benzocaine, 3 to 7.5 percent in a water-soluble base.

(2) Lidocaine in a metered spray with approximately 10 milligrams per spray.

(b) [Reserved]

Subpart C—Labeling

§ 348.50 Labeling of external analgesic drug products.

(a) *Statement of identity*. The labeling of the product contains the established name of the drug, if any, and identifies the product as follows:

(1) *For products containing any ingredient identified in § 348.10(a)*. ''Male genital desensitizer.''

(2) [Reserved]

(b) *Indications*. The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in paragraph (b) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For products containing any ingredient identified in § 348.10(a)*. (i) ''Helps in the prevention of premature ejaculation.''

(ii) ''For temporary male genital desensitization, helping to slow the onset of ejaculation.''

(iii) ''Helps in temporarily'' (select one of the following: ''retarding the onset of,'' ''slowing the onset of,'' or ''prolonging the time until'') followed by ''ejaculation.''

(iv) ''For reducing oversensitivity in the male in advance of intercourse.''

(2) [Reserved]

(c) *Warnings*. The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in § 348.10(a)*. (i) ''Premature ejaculation may be due to a condition requiring medical supervision. If this product, used as directed, does not provide relief, discontinue use and consult a doctor.''

(ii) ''Avoid contact with the eyes.''

(iii) ''If you or your partner develop a rash or irritation, such as burning or itching, discontinue use. If symptoms persist, consult a doctor.''

(2) [Reserved]

(d) *Directions*. The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing any ingredient identified in § 348.10(a)*—(i) *For products containing benzocaine identified in § 348.10(a)(1)*. ''Apply a small amount to

head and shaft of penis before intercourse, or use as directed by a doctor. Wash product off after intercourse.''

(ii) *For products containing lidocaine identified in §348.10(a)(2).* ''Apply 3 or more sprays, not to exceed 10, to head and shaft of penis before intercourse, or use as directed by a doctor. Wash product off after intercourse.''

(2) [Reserved]

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

PART 349—OPHTHALMIC DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
349.1 Scope.
349.3 Definitions.

Subpart B—Active Ingredients

349.10 Ophthalmic astringent.
349.12 Ophthalmic demulcents.
349.14 Ophthalmic emollients.
349.16 Ophthalmic hypertonicity agent.
349.18 Ophthalmic vasoconstrictors.
349.20 Eyewashes.
349.30 Permitted combinations of active ingredients.

Subpart C—Labeling

349.50 Labeling of ophthalmic drug products.
349.55 Labeling of ophthalmic astringent drug products.
349.60 Labeling of ophthalmic demulcent drug products.
349.65 Labeling of ophthalmic emollient drug products.
349.70 Labeling of ophthalmic hypertonicity drug products.
349.75 Labeling of ophthalmic vasoconstrictor drug products.
349.78 Labeling of eyewash drug products.
349.79 Labeling of permitted combinations of active ingredients.
349.80 Professional labeling.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 53 FR 7090, Mar. 4, 1988, unless otherwise noted.

Subpart A—General Provisions

§349.1 Scope.

(a) An over-the-counter ophthalmic drug product in a form suitable for topical administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this part and each of the general conditions established in §330.1.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§349.3 Definitions.

As used in this part:

(a) *Ophthalmic drug product.* A drug product, which should be sterile in accordance with §200.50, to be applied to the eyelid or instilled in the eye.

(b) *Astringent.* A locally acting pharmacologic agent which, by precipitating protein, helps to clear mucus from the outer surface of the eye.

(c) *Buffering agent.* A substance which stabilizes the pH of solutions against changes produced by introduction of acids or bases from such sources as drugs, body fluids, tears, etc.

(d) *Demulcent.* An agent, usually a water-soluble polymer, which is applied topically to the eye to protect and lubricate mucous membrane surfaces and relieve dryness and irritation.

(e) *Emollient.* An agent, usually a fat or oil, which is applied locally to eyelids to protect or soften tissues and to prevent drying and cracking.

(f) *Eyewash, eye lotion, irrigating solution.* A sterile aqueous solution intended for washing, bathing, or flushing the eye.

(g) *Hypertonicity agent.* An agent which exerts an osmotic gradient greater than that present in body tissues and fluids, so that water is drawn from the body tissues and fluids across semipermeable membranes. Applied topically to the eye, a hypertonicity agent creates an osmotic gradient which draws water out of the cornea.

(h) *Isotonicity.* A state or quality in which the osmotic pressure in two fluids is equal.

(i) *Vasoconstrictor.* A pharmacologic agent which, when applied topically to the mucous membranes of the eye,

causes transient constriction of conjunctival blood vessels.

Subpart B—Active Ingredients

§ 349.10 Ophthalmic astringent.

The active ingredient and its concentration in the product is as follows: Zinc sulfate, 0.25 percent.

§ 349.12 Ophthalmic demulcents.

The active ingredients of the product consist of any of the following, within the established concentrations for each ingredient:

(a) Cellulose derivatives:

(1) Carboxymethylcellulose sodium, 0.2 to 2.5 percent.

(2) Hydroxyethyl cellulose, 0.2 to 2.5 percent.

(3) Hydroxypropyl methylcellulose, 0.2 to 2.5 percent.

(4) Methylcellulose, 0.2 to 2.5 percent.

(b) Dextran 70, 0.1 percent when used with another polymeric demulcent agent in this section.

(c) Gelatin, 0.01 percent.

(d) Polyols, liquid:

(1) Glycerin, 0.2 to 1 percent.

(2) Polyethylene glycol 300, 0.2 to 1 percent.

(3) Polyethylene glycol 400, 0.2 to 1 percent.

(4) Polysorbate 80, 0.2 to 1 percent.

(5) Propylene glycol, 0.2 to 1 percent.

(e) Polyvinyl alcohol, 0.1 to 4 percent.

(f) Povidone, 0.1 to 2 percent.

§ 349.14 Ophthalmic emollients.

The active ingredients of the product consist of any of the following:

(a) Lanolin preparations:

(1) Anhydrous lanolin, 1 to 10 percent in combination with one or more oleaginous emollient agents included in the monograph.

(2) Lanolin, 1 to 10 percent in combination with one or more oleaginous emollient agents included in the monograph.

(b) Oleaginous ingredients:

(1) Light mineral oil, up to 50 percent in combination with one or more other emollient agents included in the monograph.

(2) Mineral oil, up to 50 percent in combination with one or more other emollient agents included in the monograph.

(3) Paraffin, up to 5 percent in combination with one or more other emollient agents included in the monograph.

(4) Petrolatum, up to 100 percent.

(5) White ointment, up to 100 percent.

(6) White petrolatum, up to 100 percent.

(7) White wax, up to 5 percent in combination with one or more other emollient agents included in the monograph.

(8) Yellow wax, up to 5 percent in combination with one or more other emollient agents included in the monograph.

§ 349.16 Ophthalmic hypertonicity agent.

The active ingredient and its concentration in the product is as follows: Sodium chloride, 2 to 5 percent.

§ 349.18 Ophthalmic vasoconstrictors.

The active ingredient of the product consists of one of the following, within the established concentration for each ingredient:

(a) Ephedrine hydrochloride, 0.123 percent.

(b) Naphazoline hydrochloride, 0.01 to 0.03 percent.

(c) Phenylephrine hydrochloride, 0.08 to 0.2 percent.

(d) Tetrahydrozoline hydrochloride, 0.01 to 0.05 percent.

§ 349.20 Eyewashes.

These products contain water, tonicity agents to establish isotonicity with tears, agents for establishing pH and buffering to achieve the same pH as tears, and a suitable preservative agent.

§ 349.30 Permitted combinations of active ingredients.

The following combinations are permitted provided each active ingredient is present within the established concentration, and the product is labeled in accordance with § 349.79.

(a) Any single ophthalmic astringent active ingredient identified in § 349.10 may be combined with any single ophthalmic vasoconstrictor active ingredient identified in § 349.18.

(b) Any two or three ophthalmic demulcent active ingredients identified in § 349.12 may be combined.

(c) Any single ophthalmic demulcent active ingredient identified in § 349.12 or any ophthalmic demulcent combination identified in paragraph (b) of this section may be combined with any single ophthalmic vasoconstrictor identified in § 349.18.

(d) Any single ophthalmic astringent active ingredient identified in § 349.10 may be combined with any single ophthalmic vasoconstrictor active ingredient identified in § 349.18 and any single ophthalmic demulcent identified in § 349.12 or ophthalmic demulcent combination identified in paragraph (b) of this section.

(e) Any two or more emollient active ingredients identified in § 349.14 may be combined as necessary to give the product proper consistency for application to the eye.

Subpart C—Labeling

§ 349.50 Labeling of ophthalmic drug products.

(a) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this part.

(b) Where applicable, indications in this part applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this part, may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) The labeling of the product contains the following warnings, under the heading ''Warnings'':

(1) *For ophthalmic drug products packaged in multi-use containers.* ''To avoid contamination, do not touch tip of container to any surface. Replace cap after using.''

(2) *For ophthalmic drug products packaged in single-use containers.* ''To avoid contamination, do not touch tip of container to any surface. Do not reuse. Once opened, discard.''

(3) *For ophthalmic drug products containing mercury compounds used as a preservative.* ''This product contains (name and quantity of mercury-containing ingredient) as a preservative. Do not use this product if you are sensitive to'' (select one of the following: ''mercury'' or ''(insert name of mercury-containing ingredient) or any other ingredient containing mercury).''

§ 349.55 Labeling of ophthalmic astringent drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as an ''astringent'' (select one of the following: ''eye'' or ''ophthalmic'') ''(insert dosage form, e.g., drops).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following phrase: ''For the temporary relief of discomfort from minor eye irritations.''

(c) *Warnings.* In addition to the warnings in § 349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for products containing any ingredient identified in § 349.10:

(1) ''If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists for more than 72 hours, discontinue use and consult a doctor.''

(2) ''If solution changes color or becomes cloudy, do not use.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Instill 1 to 2 drops in the affected eye(s) up to four times daily.

§ 349.60 Labeling of ophthalmic demulcent drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug(s), if any, and identifies the product as a ''lubricant'' or ''demulcent (lubricant)'' (select one of the following: ''eye'' or ''ophthalmic'') ''(insert dosage form, e.g., drops).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' one or more of the following phrases:

(1) ''For the temporary relief of burning and irritation due to dryness of the eye.''

(2) ''For the temporary relief of discomfort due to minor irritations of the eye or to exposure to wind or sun.''

(3) ''For use as a protectant against further irritation or to relieve dryness of the eye.''

(4) ''For use as a lubricant to prevent further irritation or to relieve dryness of the eye.''

(c) *Warnings.* In addition to the warnings in § 349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for products containing any ingredient identified in § 349.12:

(1) ''If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists for more than 72 hours, discontinue use and consult a doctor.''

(2) ''If solution changes color or becomes cloudy, do not use.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Instill 1 or 2 drops in the affected eye(s) as needed.

§ 349.65 Labeling of ophthalmic emollient drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug(s), if any, and identifies the product as a ''lubricant'' or ''emollient (lubricant)'' (select one of the following: ''eye'' or ''ophthalmic'') ''(insert dosage form, e.g., ointment).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' one or more of the following phrases:

(1) ''For the temporary relief of burning and irritation due to dryness of the eye.''

(2) ''For the temporary relief of discomfort due to minor irritations of the eye or to exposure to wind or sun.''

(3) ''For use as a protectant against further irritation or to relieve dryness of the eye.''

(4) ''For use as a lubricant to prevent further irritation or to relieve dryness of the eye.''

(c) *Warnings.* In addition to the warnings in § 349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for products containing any ingredient identified in § 349.14: ''If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists for more than 72 hours, discontinue use and consult a doctor.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Pull down the lower lid of the affected eye and apply a small amount (one-fourth inch) of ointment to the inside of the eyelid.

§ 349.70 Labeling of ophthalmic hypertonicity drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''hypertonicity'' (select one of the following: ''eye'' or ''ophthalmic'') ''(insert dosage form, e.g., drops).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following phrase: ''For the temporary relief of corneal edema.''

(c) *Warnings.* In addition to the warnings in § 349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for products containing any ingredient identified in § 349.16:

(1) ''Do not use this product except under the advice and supervision of a doctor. If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists, consult a doctor.''

(2) ''This product may cause temporary burning and irritation on being instilled into the eye.''

(3) ''If solution changes color or becomes cloudy, do not use.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Instill 1 or 2 drops in the affected eye(s) every 3 or 4 hours, or as directed by a doctor.

§349.75 Labeling of ophthalmic vasoconstrictor drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug(s), if any, and identifies the product as a ''redness reliever'' or ''vasoconstrictor (redness reliever)'' (select one of the following: ''eye'' or ''ophthalmic'') ''(insert dosage form, e.g., drops).''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following phrase: ''Relieves redness of the eye due to minor eye irritations.''

(c) *Warnings.* In addition to the warnings in §349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for products containing any ingredient identified in §349.18:

(1) ''If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists for more than 72 hours, discontinue use and consult a doctor.''

(2) ''If you have glaucoma, do not use this product except under the advice and supervision of a doctor.''

(3) ''Overuse of this product may produce increased redness of the eye.''

(4) ''If solution changes color or becomes cloudy, do not use.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'': Instill 1 to 2 drops in the affected eye(s) up to four times daily.

§349.78 Labeling of eyewash drug products.

(a) *Statement of identity.* The labeling of the product identifies the product with one or more of the following terms: ''eyewash,'' ''eye lotion,'' or ''eye irrigating solution.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' one of the following phrases:

(1) ''For'' (select one of the following: ''flushing,'' ''irrigating,'' ''cleansing,'' ''washing,'' or ''bathing'') ''the eye to remove'' (select one or more of the following: ''loose foreign material,'' ''air pollutants (smog or pollen),'' or ''chlorinated water'').

(2) ''For'' (select one of the following: ''flushing,'' ''irrigating,'' ''cleansing,'' ''washing,'' or ''bathing'') ''the eye to help relieve'' (select one or more of the following: ''irritation,'' ''discomfort,'' ''burning,'' ''stinging,'' ''smarting,'' or ''itching'') ''by removing'' (select one or more of the following: ''loose foreign material,'' ''air pollutants (smog or pollen),'' or ''chlorinated water'').

(c) *Warnings.* In addition to the warnings in §349.50, the labeling of the product contains the following warnings under the heading ''Warnings'' for all eyewash products:

(1) ''If you experience eye pain, changes in vision, continued redness or irritation of the eye, or if the condition worsens or persists, consult a doctor.''

(2) ''Obtain immediate medical treatment for all open wounds in or near the eyes.''

(3) ''If solution changes color or becomes cloudy, do not use.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For eyewash products intended for use with an eyecup.* Rinse cup with clean water immediately before each use. Avoid contamination of rim and inside surfaces of cup. Fill cup half full and apply the cup to the affected eye, pressing tightly to prevent the escape of the liquid, and tilt the head backward. Open eyelids wide and rotate eyeball to ensure thorough bathing with the wash or lotion. Rinse cup with clean water after each use.

(2) *For eyewash products intended for use with a nozzle applicator.* Flush the affected eye as needed, controlling the rate of flow of solution by pressure on the bottle.

§349.79 Labeling of permitted combinations of active ingredients.

Statements of identity, indications, warnings, and directions for use, respectively, applicable to each ingredient in the product may be combined to eliminate duplicative words or phrases so that the resulting information is clear and understandable.

(a) *Statement of identity.* For a combination drug product that has an established name, the labeling of the product states the established name of

the combination drug product, followed by the statement of identity for each ingredient in the combination, as established in the statement of identity sections of this part. For a combination drug product that does not have an established name, the labeling of the product states the statement of identity for each ingredient in the combination, as established in the statement of identity sections of this part.

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the indication(s) for each ingredient in the combination, as established in the indications sections of this part.

(c) *Warnings.* The labeling of the product states, under the heading ''Warnings,'' the warning(s) for each ingredient in the combination, as established in the warnings sections of this part.

(d) *Directions.* The labeling of the product states, under the heading ''Directions,'' directions that conform to the directions established for each ingredient in the directions sections of this part. When the time intervals or age limitations for administration of the individual ingredients differ, the directions for the combination product may not exceed any maximum dosage limits established for the individual ingredients in the applicable OTC drug monograph.

§ 349.80 Professional labeling.

The labeling of any OTC ophthalmic demulcent drug product provided to health professionals (but not to the general public) may contain instructions for the use of these products in professional eye examinations (i.e. gonioscopy, electroretinography).

PART 355—ANTICARIES DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A—General Provisions

Sec.
355.1 Scope.
355.3 Definitions.

Subpart B—Active Ingredients

355.10 Anticaries active ingredients.
355.20 Packaging conditions.

Subpart C—Labeling

355.50 Labeling of anticaries drug products.
355.55 Principal display panel of all fluoride rinse drug products.
335.60 Professional labeling.

Subpart D—Testing Procedures

355.70 Testing procedures for fluoride dentifrice drug products.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 60 FR 52507, Oct. 6, 1995, unless otherwise noted.

Subpart A—General Provisions

§ 355.1 Scope.

(a) An over-the-counter anticaries drug product in a form suitable for topical administration to the teeth is generally recognized as safe and effective and is not misbranded if it meets each condition in this part and each general condition established in § 330.1 of this chapter.

(b) References in this part to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 355.3 Definitions.

As used in this part:

(a) *Abrasive.* Solid materials that are added to dentifrices to facilitate mechanical removal of dental plaque, debris, and stain from tooth surfaces.

(b) *Anhydrous glycerin.* An ingredient that may be prepared by heating glycerin U.S.P. at 150 –C for 2 hours to drive off the moisture content.

(c) *Anticaries drug.* A drug that aids in the prevention and prophylactic treatment of dental cavities (decay, caries).

(d) *Dental caries.* A disease of calcified tissues of teeth characterized by demineralization of the inorganic portion and destruction of the organic matrix.

(e) *Dentifrice.* An abrasive-containing dosage form (gel, paste, or powder) for delivering an anticaries drug to the teeth.

(f) *Fluoride.* The inorganic form of the chemical element fluorine in combination with other elements.

(g) *Fluoride ion.* The negatively charged atom of the chemical element fluorine.

(h) *Fluoride supplement.* A special treatment rinse dosage form that is intended to be swallowed, and is promoted to health professionals for use in areas where the water supply contains 0 to 0.7 parts per million (ppm) fluoride ion.

(i) *Preventive treatment gel.* A dosage form for delivering an anticaries drug to the teeth. Preventive treatment gels are formulated in an anhydrous glycerin base with suitable thickening agents included to adjust viscosity. Preventive treatment gels do not contain abrasives.

(j) *Treatment rinse.* A liquid dosage form for delivering an anticaries drug to the teeth.

(k) *Treatment rinse concentrated solution.* A fluoride treatment rinse in a concentrated form to be mixed with water before using to result in the appropriate fluoride concentration specified in the monograph.

(l) *Treatment rinse effervescent tablets.* A fluoride treatment rinse prepared by adding an effervescent tablet (a concentrated solid dosage form) to water before using to result in the appropriate fluoride concentration specified in the monograph.

(m) *Treatment rinse powder.* A fluoride treatment rinse prepared by adding the powder (a concentrated solid dosage form) to water before using to result in the appropriate fluoride concentration specified in the monograph.

[60 FR 52507, Oct. 6, 1995, as amended at 61 FR 52286, Oct. 7, 1996]

Subpart B—Active Ingredients

§ 355.10 Anticaries active ingredients.

The active ingredient of the product consists of any of the following when used in the concentration and dosage form established for each ingredient:

(a) *Sodium fluoride*—(1) *Dentifrices containing 850 to 1,150 ppm theoretical total fluorine in a gel or paste dosage form.* Sodium fluoride 0.188 to 0.254 percent with an available fluoride ion concentration ≥ 650 parts per million (ppm).

(2) *Dentifrices containing 850 to 1,150 ppm theoretical total fluorine in a powdered dosage form.* Sodium fluoride 0.188 to 0.254 percent with an available fluoride ion concentration of ≥ 850 ppm for products containing the abrasive sodium bicarbonate and a poured-bulk density of 1.0 to 1.2 grams per milliliter.

(3) *Treatment rinses.* (i) An aqueous solution of acidulated phosphate fluoride derived from sodium fluoride acidulated with a mixture of sodium phosphate, monobasic, and phosphoric acid to a level of 0.1 molar phosphate ion and a pH of 3.0 to 4.5 and which yields an effective fluoride ion concentration of 0.02 percent.

(ii) An aqueous solution of acidulated phosphate fluoride derived from sodium fluoride acidulated with a mixture of sodium phosphate, dibasic, and phosphoric acid to a pH of 3.5 and which yields an effective fluoride ion concentration of 0.01 percent.

(iii) Sodium fluoride 0.02 percent aqueous solution with a pH of approximately 7.

(iv) Sodium fluoride 0.05 percent aqueous solution with a pH of approximately 7.

(v) Sodium fluoride concentrate containing adequate directions for mixing with water before using to result in a 0.02-percent or 0.05-percent aqueous solution with a pH of approximately 7.

(b) *Sodium monofluorophosphate*—(1) *Dentifrices containing 850 to 1,150 ppm theoretical total fluorine in a gel or paste dosage form.* Sodium monofluorophosphate 0.654 to 0.884 percent with an available fluoride ion concentration (consisting of $PO_3F^=$ and F^- combined) ≥ 800 ppm.

(2) *Dentifrices containing 1,500 ppm theoretical total fluorine in a gel or paste dosage form.* Sodium monofluorophosphate 1.153 percent with an available fluoride ion concentration (consisting of $PO_3F^=$ and F^- combined) ≥ 1,275 ppm.

(c) *Stannous fluoride*—(1) *Dentifrices containing 850 to 1,150 ppm theoretical total fluorine in a gel or paste dosage form.* (i) Stannous fluoride 0.351 to 0.474 percent with an available fluoride ion concentration ≥ 700 ppm for products containing abrasives other than calcium pyrophosphate.

(ii) Stannous fluoride 0.351 to 0.474 percent with an available fluoride ion concentration ≥ 290 ppm for products

containing the abrasive calcium pyrophosphate.

(2) *Preventive treatment gel.* Stannous fluoride 0.4 percent in an anhydrous glycerin gel, made from anhydrous glycerin and the addition of suitable thickening agents to adjust viscosity.

(3) *Treatment rinse.* Stannous fluoride concentrate marketed in a stable form and containing adequate directions for mixing with water immediately before using to result in a 0.1-percent aqueous solution.

[60 FR 52507, Oct. 6, 1995, as amended at 61 FR 52286, Oct. 7, 1996]

§ 355.20 Packaging conditions.

(a) *Package size limitation.* Due to the toxicity associated with fluoride active ingredients, the following package size limitations are required for anticaries drug products:

(1) *Dentifrices.* Dentifrice (toothpastes and tooth powders) packages shall not contain more than 276 milligrams (mg) total fluorine per package.

(2) *Preventive treatment gels and treatment rinses.* Preventive treatment gel and treatment rinse packages shall not contain more than 120 mg total fluorine per package.

(3) *Exception.* Package size limitations do not apply to anticaries drug products marketed for professional office use only and labeled in accord with § 355.60.

(b) *Tight container packaging.* To minimize moisture contamination, all fluoride powdered dentifrices shall be packaged in a tight container as defined as a container that protects the contents from contamination by extraneous liquids, solids, or vapors, from loss of the article, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and distribution, and is capable of tight reclosure.

Subpart C—Labeling

§ 355.50 Labeling of anticaries drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as: (select one or both of the following: 'anticavity' or 'fluoride') (select one of the following as appropriate: ''dentifrice,'' ''toothpaste,'' ''tooth polish,'' ''tooth powder;'' (optional: ''dental'') ''preventive treatment gel;'' or (optional: ''treatment'' or ''dental'')) (select one of the following: ''rinse,'' ''concentrated solution,'' ''rinse powder,'' or ''rinse effervescent tablets''). The word ''mouthwash'' may be substituted for the word ''rinse'' in this statement of identity if the product also has a cosmetic use, as defined in section 201(i) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 321(i)).

(b) *Indication.* The labeling of the product states, under the heading ''Indication,'' the following: ''Aids in the prevention of dental (select one of the following: ''cavities,'' ''decay,'' ''caries (decay),'' or ''caries (cavities)''). Other truthful and nonmisleading statements, describing only the indication for use that has been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warning.* The labeling of the product contains the following warning under the heading ''Warning'':

(1) *For all fluoride dentifrice (gel, paste, and powder) products.* ''Keep out of the reach of children under 6 years of age. If you accidentally swallow more than used for brushing, seek professional assistance or contact a Poison Control Center immediately.'' These warnings shall be used in place of the general warning statements required by § 330.1(g) of this chapter.

(2) *For all fluoride rinse and preventive treatment gel products.* ''Keep this and all drugs out of the reach of children. If you accidentally swallow more than used for'' (select appropriate word: ''brushing'' or ''rinsing''), ''seek professional assistance or contact a Poison Control Center immediately.'' These warnings shall be used in place of the general warning statements required by § 330.1(g) of this chapter.

(d) *Directions.* The labeling of the product contains the following statements under the heading ''Directions'':

(1) *For anticaries dentifrice products*—(i) *Gel or paste dosage form with a theoretical total fluorine concentration of 850 to 1,150 ppm identified in § 355.10(a)(1), (b)(1), and (c)(1).* Adults and children 2 years of age and older: Brush teeth thoroughly, preferably after each meal or at least twice a day, or as directed by a dentist or doctor. Instruct children under 6 years of age in good brushing and rinsing habits (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 2 years of age: Consult a dentist or doctor.

(ii) *Gel or paste dosage form with a theoretical total fluorine concentration of 1,500 ppm identified in § 355.10(b)(2).* Adults and children 6 years of age and older: Brush teeth thoroughly, preferably after each meal or at least twice a day, or as directed by a dentist or doctor. Instruct children under 12 years of age in good brushing and rinsing habits (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 6 years of age: Do not use unless directed by a dentist or doctor.

(iii) *Powdered dosage form with a theoretical total fluorine concentration of 850 to 1,150 ppm identified in § 355.10(a)(2).* Adults and children 6 years of age and older: Apply powder to a wet toothbrush; completely cover all bristles. Brush for at least 30 seconds. Reapply powder as before and brush again. Rinse and spit out thoroughly. Brush teeth, preferably after each meal or at least twice a day, or as directed by a dentist or doctor. Instruct children under 12 years of age in good brushing and rinsing habits (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 6 years of age: Do not use unless directed by a dentist or doctor.

(2) *For anticaries treatment rinse products*—(i) *For acidulated phosphate fluoride solution containing 0.02 percent fluoride ion, sodium fluoride 0.05 percent, sodium fluoride concentrate, and stannous fluoride concentrate identified in § 355.10(a)(3)(i), (a)(3)(iv), (a)(3)(v), and (c)(3).* Adults and children 6 years of age and older: Use once a day after brushing your teeth with a toothpaste. Vigorously swish 10 milliliters of rinse between your teeth for 1 minute and then spit out. Do not swallow the rinse. Do not eat or drink for 30 minutes after rinsing. Instruct children under 12 years of age in good rinsing habits (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 6 years of age: Consult a dentist or doctor.

(ii) *For acidulated phosphate fluoride solution containing 0.01 percent fluoride ion and sodium fluoride 0.02 percent aqueous solution identified in § 355.10(a)(3)(ii) and (a)(3)(iii).* Adults and children 6 years of age and older: Use twice a day after brushing your teeth with a toothpaste. Vigorously swish 10 milliliters of rinse between your teeth for 1 minute and then spit out. Do not swallow the rinse. Do not eat or drink for 30 minutes after rinsing. Instruct children under 12 years of age in good rinsing habits (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 6 years of age: consult a dentist or doctor.

(3) *For stannous fluoride treatment rinse products.* (i) ''Use immediately after preparing the rinse.''

(ii) *For powder or effervescent tablets used to prepare treatment rinses.* ''Do not use as a rinse until all the'' (select one of the following: ''powder'' or ''tablet'') ''has dissolved.''

(4) *For anticaries preventive treatment gel products.* Adults and children 6 years of age and older: Use once a day after brushing your teeth with a toothpaste. Apply the gel to your teeth and brush thoroughly. Allow the gel to remain on your teeth for 1 minute and then spit out. Do not swallow the gel. Do not eat or drink for 30 minutes after brushing. Instruct children under 12 years of age in the use of this product (to minimize swallowing). Supervise children as necessary until capable of using without supervision. Children under 6 years of age: consult a dentist or doctor.

(5) *For all concentrated treatment rinse solutions, powders, and effervescent tablets.* The following statement shall appear as the first statement under directions: ''Do not use before mixing with water.''

(e) *Additional labeling statements for anticaries drug products.* The following statements need not appear under warnings, but are required to appear on the label of anticaries drugs products as applicable.

(1) *For all preventive treatment gels.* ''This is a(n)'' (select one or both of the following: ''anticavity'' or ''fluoride'') ''preventive treatment gel, not a toothpaste. Read directions carefully before using.''

(2) *For all stannous fluoride treatment rinse, preventive treatment gel, and dentifrice products.* ''This product may produce surface staining of the teeth. Adequate toothbrushing may prevent these stains which are not harmful or permanent and may be removed by your dentist.''

(f) *Optional additional labeling statements*—(1) *For fluoride treatment rinses and preventive treatment gels.* The following labeling statement may appear in the required boxed area designated ''APPROVED USES'': ''The combined daily use of a fluoride preventive treatment'' (select one of the following: ''rinse'' or ''gel'') ''and a fluoride toothpaste can help reduce the incidence of dental cavities.''

(2) *For dentifrice products containing 1,500 ppm theoretical total fluorine.* ''Adults and children over 6 years of age may wish to use this extra-strength fluoride dentifrice if they reside in a nonfluoridated area or if they have a greater tendency to develop cavities.''

[60 FR 52507, Oct. 6, 1995; 60 FR 57927, Nov. 24, 1995; 61 FR 51187, Oct. 7, 1996]

§ 355.55 Principal display panel of all fluoride rinse drug products.

In addition to the statement of identity required in § 355.50, the following statement shall be prominently placed on the principal display panel: ''IMPORTANT: Read directions for proper use.''

§ 355.60 Professional labeling.

(a) The labeling for anticaries fluoride treatment rinses identified in § 355.10(a)(3) and (c)(3) that are specially formulated so they may be swallowed (fluoride supplements) and are provided to health professionals (but not to the general public) may contain the following additional dosage information: Children 3 to under 14 years of age: As a supplement in areas where the water supply is nonfluoridated (less than 0.3 parts per million (ppm)), clean the teeth with a toothpaste and rinse with 5 milliliters (mL) of 0.02 percent or 10 mL of 0.01 percent fluoride ion rinse daily, then swallow. When the water supply contains 0.3 to 0.7 ppm fluoride ion, reduce the dose to 2.5 mL of 0.02 percent or 5 mL of 0.01 percent fluoride ion rinse daily.

(b) The labeling for products marketed to health to health professionals in package sizes larger than those specified in § 355.20 shall include the statements: ''For Professional Office Use Only'' and ''This product is not intended for home or unsupervised consumer use.''

Subpart D—Testing Procedures

§ 355.70 Testing procedures for fluoride dentifrice drug products.

(a) A fluoride dentifrice drug product shall meet the biological test requirements for animal caries reduction and one of the following tests: Enamel solubility reduction or fluoride enamel uptake. The testing procedures for these biological tests are labeled *Biological Testing Procedures for Fluoride Dentifrices*; these testing procedures are on file under Docket No. 80N–0042 in the Dockets Management Branch (HFA–305), Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857, and are available on request to that office.

(b) The United States Pharmacopeia fluoride dentifrice reference standards along with reference standard stability profiles (total fluoride, available fluoride ion, pH, and specific gravity) required to be used in the biological tests are available to any purchaser upon written request to the United States Pharmacopeial Convention, Inc., 1260

Twinbrook Parkway, Rockville, MD 20852.

(c) Alternative testing procedures may be used. Any proposed modification or alternative testing procedures shall be submitted as a petition in accord with § 10.30 of this chapter. The petition should contain data to support the modification or data demonstrating that an alternative testing procedure provides results of equivalent accuracy. All information submitted will be subjected to the disclosure rules in part 20 of this chapter.

PART 357—MISCELLANEOUS INTERNAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A [Reserved]

Subpart B—Anthelmintic Drug Products

Sec.
357.101 Scope.
357.103 Definition.
357.110 Anthelmintic active ingredient.
357.150 Labeling of anthelmintic drug products.
357.152 Package inserts for anthelmintic drug products.
357.180 Professional labeling.

Subpart C—Cholecystokinetic Drug Products

357.201 Scope.
357.203 Definition.
357.210 Cholecystokinetic active ingredients.
357.250 Labeling of cholecystokinetic drug products.
357.280 Professional labeling.

Subparts D–H [Reserved]

Subpart I—Deodorant Drug Products for Internal Use

357.801 Scope.
357.803 Definitions.
357.810 Active ingredients for deodorant drug products for internal use.
357.850 Labeling of deodorant drug products for internal use.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

Subpart A [Reserved]

Subpart B—Anthelmintic Drug Products

SOURCE: 51 FR 27759, Aug. 1, 1986, unless otherwise noted.

§ 357.101 Scope.

(a) An over-the-counter anthelmintic drug product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this subpart and each general condition established in § 330.1.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 357.103 Definition.

As used in this subpart:

Anthelmintic. An agent that is destructive to worms.

§ 357.110 Anthelmintic active ingredient.

The active ingredient of the product is pyrantel pamoate when used within the dosage limits established in § 357.150(d)(1).

§ 357.150 Labeling of anthelmintic drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''pinworm treatment.''

(b) *Indication.* The labeling of the product states, under the heading ''Indication,'' the following: ''For the treatment of pinworms.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''Abdominal cramps, nausea, vomiting, diarrhea, headache, or dizziness sometimes occur after taking this drug. If any of these conditions persist consult a doctor.''

(2) ''If you are pregnant or have liver disease, do not take this product unless directed by a doctor.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) Adults, children 12 years of age and over, and children 2 years to under 12 years of age: Oral dosage is a single dose of 5 milligrams of pyrantel base per pound, or 11 milligrams per kilogram, of body weight not to exceed 1 gram. Dosing information should be converted to easily understood directions for the consumer using the following dosage schedule:

Weight	Dosage (taken as a single dose) [1]
Less than 25 pounds or under 2 years old.	Do not use unless directed by a doctor.
25 to 37 pounds	125 milligrams.
38 to 62 pounds	250 milligrams.
63 to 87 pounds	375 milligrams.
88 to 112 pounds	500 milligrams.
113 to 137 pounds	625 milligrams.
138 to 162 pounds	750 milligrams.
163 to 187 pounds	875 milligrams.
188 pounds and over	1,000 milligrams.

[1] Depending on the product, the label should state the quantity of drug as a liquid measurement (e.g., teaspoonsful) or as the number of dosage units (e.g., tablets) to be taken for the varying body weights. (If appropriate, it is recommended that a measuring cup graduated by body weight and/or liquid measurement be provided with the product.) Manufacturers should present this information as appropriate for their product and may vary the format of this chart as necessary.

(2) ''Read package insert carefully before taking this medication. Take only according to directions and do not exceed the recommended dosage unless directed by a doctor. Medication should only be taken on time as a single dose; do not repeat treatment unless directed by a doctor. When one individual in a household has pinworms, the entire household should be treated unless otherwise advised. See Warnings. If any worms other than pinworms are present before or after treatment, consult a doctor. If any symptoms or pinworms are still present after treatment, consult a doctor.

(3) ''This product can be taken any time of day, with or without meals. It may be taken alone or with milk or fruit juice. Use of a laxative is not necessary prior to, during, or after medication.''

(e) *Optional wording.* The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[51 FR 27759, Aug. 1, 1986; 52 FR 7831, Mar. 13, 1987, as amended at 53 FR 35810, Sept. 15, 1988]

§ 357.152 Package inserts for anthelmintic drug products.

The labeling of the product contains a consumer package insert which includes the following information:

(a) A discussion of the symptoms suggestive of pinworm infestation, including a statement that pinworms must be visually identified before taking this medication.

(b) A detailed description of how to find and identify the pinworm.

(c) A commentary on the life cycle of the pinworm.

(d) A commentary on the ways in which pinworms may be spread from person to person and hygienic procedures to follow to avoid such spreading.

(e) The appropriate labeling information contained in § 357.150

(Collection of information requirement approved by the Office of Management and Budget under control number 0910–0232)

[51 FR 27759, Aug. 1, 1986, as amended at 52 FR 2515, Jan 23, 1987]

§ 357.180 Professional labeling.

The labeling provided to health professionals (but not to the general public) may contain an additional indication: ''For the treatment of common roundworm infestation.''

Subpart C—Cholecystokinetic Drug Products

§ 357.201 Scope.

(a) An over-the-counter cholecystokinetic drug product in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart

in addition to each of the general conditions established in § 330.1.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

[48 FR 27005, June 10, 1983]

§ 357.203 Definition.

As used in this subpart:

Cholecystokinetic drug product. A drug product that causes contraction of the gallbladder and is used during the course of diagnostic gallbladder studies (cholecystography).

[48 FR 27005, June 10, 1983]

§ 357.210 Cholecystokinetic active ingredients.

The active ingredient of the product consists of any of the following when used within the specified concentration and dosage form established for each ingredient:

(a) 50-percent aqueous emulsion of corn oil.

(b) Hydrogenated soybean oil in a suitable, water-dispersible powder. The hydrogenated soybean oil is food-grade, partially hydrogenated with a melting point of 41 to 43.5 ° C, an iodine value of 65 to 69, and a fatty acid composition as follows:

Fatty acid	Percent composition
Myristic acid	0.1
Palmitic acid	10.0
Palmitoleic acid	0.1
Stearic acid	13.5
Oleic acid	72.0
Linoleic acid	3.8
Linolenic acid	0.1
Arachidic acid	0.5
Behenic acid	0.2

[54 FR 8321, Feb. 28, 1989]

§ 357.250 Labeling of cholecystokinetic drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''gallbladder diagnostic agent.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''For the contraction of the gallbladder during diagnostic gallbladder studies.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in this paragraph (b), may also be used, as provided in § 330.1(c)(2), subject to the provisions of section 502 of the act relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* [Reserved]

(d) *Directions.* The labeling of the product contains the following statements under the heading ''Directions'':

(1) ''Take only when instructed by a doctor:''

(2) *For products containing 50-percent aqueous emulsion of corn oil.*

(i) ''Shake well before using.''

(ii) Oral dosage is 60 milliliters 20 minutes before diagnostic gallbladder x-ray or as directed by a doctor.

(3) *For products containing hydrogeneated soybean oil.* Oral dosage is 12.4 grams in a suitable, water-dispersible powder in 2 to 3 ounces of water. Stir briskly to prepare a suspension before using. Drink 20 minutes before diagnostic gallbladder x-ray or as directed by a doctor.

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[48 FR 27005, June 10, 1983, as amended at 51 FR 16267, May 1, 1986; 52 FR 7830, Mar. 13, 1987; 54 FR 8321, Feb. 28, 1989]

§ 357.280 Professional labeling.

The labeling provided to health professionals (but not to the general public) may contain the following information for ingredients identified in § 357.210: *Indication.* ''For visualization of biliary ducts during cholecystography.''

[54 FR 8321, Feb. 28, 1989]

Subparts D–H [Reserved]

Subpart I—Deodorant Drug Products for Internal Use

SOURCE: 55 FR 19865, May 11, 1990, unless otherwise noted.

§ 357.801 Scope.

(a) An over-the-counter deodorant drug product for internal use in a form suitable for oral administration is generally recognized as safe and effective and is not misbranded if it meets each condition in this subpart and each general condition established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 357.803 Definitions.

As used in this subpart:

(a) *Colostomy.* An external operative opening of the colon.

(b) *Deodorant for internal use.* An ingredient taken internally to reduce odors arising from conditions such as colostomies, ileostomies, or fecal incontinence.

(c) *Ileostomy.* An external operative opening from the ileum.

(d) *Incontinence.* An inability to retain urine or feces.

§ 357.810 Active ingredients for deodorant drug products for internal use.

The active ingredient of the product consists of either of the following when used within the dosage limits established for each ingredient in § 357.850(d):

(a) Bismuth subgallate.

(b) Chlorophyllin copper complex.

§ 357.850 Labeling of deodorant drug products for internal use.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''deodorant for internal use'' or as a ''colostomy or ileostomy deodorant.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in paragraph (b) of this section as appropriate. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraph (b) of this section may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) *For products containing bismuth subgallate identified in § 357.810(a).* ''An aid to reduce odor from a colostomy or ileostomy.''

(2) *For products containing chlorophyllin copper complex identified in § 357.810(b).* (i) ''An aid to reduce odor from a colostomy or ileostomy.''

(ii) ''An aid to reduce fecal odor due to incontinence.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'': (1) *For products containing chlorophyllin copper complex identified in § 357.810(b).* (i) ''If cramps or diarrhea occurs, reduce the dosage. If symptoms persist, consult your doctor.''

(ii) The warning required by § 330.1(g) of this chapter concerning overdose is not required on products containing chlorophyllin copper complex identified in § 357.810(b).

(2) [Reserved]

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions.''

(1) *For products containing bismuth subgallate identified in § 357.810(a).* Adults and children 12 years of age and over: Oral dosage is 200 to 400 milligrams up to 4 times daily. Children under 12 years of age: consult a doctor.

(2) *For products containing chlorophyllin copper complex identified in § 357.810(b).* Adults and children 12 years of age and over: Oral dosage is 100 to 200 milligrams daily in divided doses as required. If odor is not controlled, take up to an additional 100 milligrams daily in divided doses as required. The smallest effective dose should be used. Do not exceed 300 milligrams daily. Children under 12 years of age: consult a doctor.

PART 358—MISCELLANEOUS EXTERNAL DRUG PRODUCTS FOR OVER-THE-COUNTER HUMAN USE

Subpart A [Reserved]

Subpart B—Wart Remover Drug Products

Sec.
358.101 Scope.
358.103 Definitions.
358.110 Wart remover active ingredients.
358.150 Labeling of wart remover drug products.

Subparts C–E [Reserved]

Subpart F—Corn and Callus Remover Drug Products

358.501 Scope.
358.503 Definitions.
358.510 Corn and callus remover active ingredients.
358.550 Labeling of corn and callus remover drug products.

Subpart G—Pediculicide Drug Products

358.601 Scope.
358.603 Definition.
358.610 Pediculicide active ingredients.
358.650 Labeling of pediculicide drug products.

Subpart H—Drug Products for the Control of Dandruff, Seborrheic Dermatitis, and Psoriasis

358.701 Scope.
358.703 Definitions.
358.710 Active ingredients for the control of dandruff, seborrheic dermatitis, or psoriasis.
358.720 Permitted combinations of active ingredients.
358.750 Labeling of drug products for the control of dandruff, seborrheic dermatitis, or psoriasis.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371.

SOURCE: 55 FR 33255, Aug. 14, 1990, unless otherwise noted.

Subpart A [Reserved]

Subpart B—Wart Remover Drug Products

§ 358.101 Scope.

(a) An over-the-counter wart remover drug product in a form suitable for topical application is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart and each of the general conditions established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 358.103 Definitions.

As used in this subpart:

(a) *Wart remover drug product.* A topical agent used for the removal of common or plantar warts.

(b) *Collodion-like vehicle.* A solution containing pyroxylin (nitrocellulose) in an appropriate nonaqueous solvent that leaves a transparent cohesive film when applied to the skin in a thin layer.

(c) *Plaster vehicle.* A fabric, plastic, or other suitable backing material in which medication is usually incorporated for topical application to the skin.

§ 358.110 Wart remover active ingredients.

The product consists of any of the following active ingredients within the specified concentration and in the dosage form established for each ingredient.

(a) Salicylic acid 12 to 40 percent in a plaster vehicle.

(b) Salicylic acid 5 to 17 percent in a collodion-like vehicle.

(c) Salicylic acid 15 percent in a karaya gum, glycol plaster vehicle.

§ 358.150 Labeling of wart remover drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''wart remover.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' any of the phrases listed in paragraph (b) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food,

Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''For the removal of common warts. The common wart is easily recognized by the rough 'cauliflower-like' appearance of the surface.''

(2) ''For the removal of plantar warts on the bottom of the foot. The plantar wart is recognized by its location only on the bottom of the foot, its tenderness, and the interruption of the footprint pattern.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in § 358.110.* (i) ''For external use only.''

(ii) ''Do not use this product on irritated skin, on any area that is infected or reddened, if you are a diabetic, or if you have poor blood circulation.''

(iii) ''If discomfort persists, see your doctor.''

(iv) ''Do not use on moles, birthmarks, warts with hair growing from them, genital warts, or warts on the face or mucous membranes.''

(2) *For any product formulated in a flammable vehicle.* (i) The labeling should contain an appropriate flammability signal word, e.g. ''extremely flammable,'' ''flammable,'' ''combustible,'' consistent with 16 CFR 1500.3(b)(10).

(ii) ''Keep away from fire or flame.''

(3) *For any product formulated in a volatile vehicle.* ''Cap bottle tightly and store at room temperature away from heat.''

(4) *For any product formulated in a collodion-like vehicle.* (i) ''If product gets into the eye, flush with water for 15 minutes.''

(ii) ''Avoid inhaling vapors.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing salicylic acid identified in § 358.110(a).* ''Wash affected area.'' (Optional: ''May soak wart in warm water for 5 minutes.'') ''Dry area thoroughly.'' (If appropriate: ''Cut plaster to fit wart.'') ''Apply medicated plaster. Repeat procedure every 48 hours as needed (until wart is removed) for up to 12 weeks.''

(2) *For products containing salicylic acid identified in § 358.110(b).* ''Wash affected area.'' (Optional: ''May soak wart in warm water for 5 minutes.'') ''Dry area thoroughly. Apply'' (select one of the following, as appropriate: ''one drop'' or ''small amount'') ''at a time with'' (select one of the following, as appropriate: ''applicator'' or ''brush'') ''to sufficiently cover each wart. Let dry. Repeat this procedure once or twice daily as needed (until wart is removed) for up to 12 weeks.''

(3) *For products containing salicylic acid identified in § 358.110(c).* ''Wash affected area.'' (Optional: ''May soak wart in warm water for 5 minutes.'') ''Dry area thoroughly. Gently smooth wart surface with emery file supplied.'' (If appropriate: ''Cut plaster to fit wart.'') ''Apply a drop of warm water to the wart, keeping the surrounding skin dry. Apply medicated plaster at bedtime and leave in place for at least 8 hours. In the morning, remove plaster and discard. Repeat procedure every 24 hours as needed (until wart is removed) for up to 12 weeks.''

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

(f) The phrase ''or podiatrist'' may be used in addition to the word ''doctor'' in any of the labeling statements in this section when a product is labeled with the indication identified in § 358.150(b)(2).

[55 FR 33255, Aug. 14, 1990; 55 FR 37403, Sept. 11, 1990, as amended at 57 FR 44495, Sept. 28, 1992; 59 FR 60317, Nov. 23, 1994]

Subparts C–E [Reserved]

Subpart F—Corn and Callus Remover Drug Products

SOURCE: 55 FR 33261, Aug. 14, 1990, unless otherwise noted.

§ 358.501 Scope.

(a) An over-the-counter corn and callus remover drug product in a form suitable for topical application is generally recognized as safe and effective

and is not misbranded if it meets each of the conditions in this subpart and each of the general conditions established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 358.503 Definitions.

As used in this subpart:

(a) *Corn and callus remover drug product.* A topical agent used for the removal of corns and calluses.

(b) *Collodion-like vehicle.* A solution containing pyroxylin (nitrocellulose) in an appropriate nonaqueous solvent that leaves a transparent cohesive film when applied to the skin in a thin layer.

(c) *Plaster vehicle.* A fabric, plastic, or other suitable backing material in which medication is usually incorporated for topical application to the skin.

§ 358.510 Corn and callus remover active ingredients.

The product consists of any of the following active ingredients within the specified concentrations and in the dosage form established for each ingredient.

(a) Salicylic acid 12 to 40 percent in a plaster vehicle.

(b) Salicylic acid 12 to 17.6 percent in a collodion-like vehicle.

§ 358.550 Labeling of corn and callus remover drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''corn and callus remover.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1) of this section and may contain the additional phrase listed in paragraph (b)(2) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) ''For the removal of corns and calluses.''

(2) In addition to the information identified in paragraph (b)(1) of this section, the labeling of the product may contain the following statement: ''Relieves pain by removing corns and calluses.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in § 358.510.* (i) ''For external use only.''

(ii) ''Do not use this product on irritated skin, on any area that is infected or reddened, if you are a diabetic, or if you have poor blood circulation.''

(iii) ''If discomfort persists, see your doctor or podiatrist.''

(2) *For any product formulated in a flammable vehicle.* (i) The labeling should contain an appropriate flammability signal word, e.g., ''extremely flammable,'' ''flammable,'' ''combustible,'' consistent with 16 CFR 1500.3(b)(10).

(ii) ''Keep away from fire or flame.''

(3) *For any product formulated in a volatile vehicle.* ''Cap bottle tightly and store at room temperature away from heat.''

(4) *For any product formulated in a collodion-like vehicle.* (i) ''If product gets into the eye, flush with water for 15 minutes.''

(ii) ''Avoid inhaling vapors.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For products containing salicylic acid identified in § 358.510(a).* ''Wash affected area and dry thoroughly.'' (If appropriate: ''Cut plaster to fit corn/callus.'') ''Apply medicated plaster. After 48 hours remove the medicated plaster. Repeat this procedure every 48 hours as needed for up to 14 days (until corn/callus is removed).'' (Optional: ''May soak corn/callus in warm water for 5 minutes to assist in removal.'')

(2) *For products containing salicylic acid identified in § 358.510(b).* ''Wash affected area and dry thoroughly. Apply'' (select one of the following, as appropriate: ''one drop'' or ''small amount'') ''at a time with'' (select one of the following, as appropriate: ''applicator'' or ''brush'') ''to sufficiently cover each corn/callus. Let dry. Repeat this procedure once or twice daily as needed for up to 14 days (until corn/callus is removed).'' (Optional: ''May soak corn/callus in warm water for 5 minutes to assist in removal.'')

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

[55 FR 33261, Aug. 14, 1990, as amended at 57 FR 44494, Sept. 28, 1992]

Subpart G—Pediculicide Drug Products

SOURCE: 58 FR 65455, Dec. 14, 1993, unless otherwise noted.

§ 358.601 Scope.

(a) An over-the-counter pediculicide drug product in a form suitable for topical application is generally recognized as safe and effective and is not misbranded if it meets each condition in this subpart and each general condition established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 358.603 Definition.

As used in this subpart:

Pediculicide drug product. A drug product for the treatment of head, pubic (crab), and body lice.

§ 358.610 Pediculicide active ingredients.

The active ingredients of the product consist of the combination of pyrethrum extract (0.17 to 0.33 percent) with piperonyl butoxide (2 to 4 percent) in a nonaerosol dosage formulation.

§ 358.650 Labeling of pediculicide drug products.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product as a ''pediculicide (lice treatment)'' or ''lice treatment.''

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the following: ''For the treatment of head, pubic (crab), and body lice.'' Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) ''Use with caution on persons allergic to ragweed.''

(2) ''For external use only. Do not use near the eyes or permit contact with mucous membranes, such as inside the nose, mouth, or vagina, as irritation may occur. Keep out of eyes when rinsing hair. Adults and children: Close eyes tightly and do not open eyes until product is rinsed out. Also, protect children's eyes with washcloth, towel or other suitable material, or by a similar method. If product gets into the eyes, immediately flush with water.''

(3) ''If skin irritation or infection is present or develops, discontinue use and consult a doctor. Consult a doctor if infestation of eyebrows or eyelashes occurs.''

(4) The word ''physician'' may be substituted for the word ''doctor'' in any of the warning statements in this paragraph.

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions'':

(1) *For all products.* ''Important: Read warnings before using.'' [sentence in all capital letters and boldface type]

(2) *For nonshampoo products.* ''Apply to affected area until all the hair is thoroughly wet with product. Allow

product to remain on area for 10 minutes but no longer. Wash area thoroughly with warm water and soap or shampoo. A fine-toothed comb or a special lice/nit removing comb may be used to help remove dead lice or their eggs (nits) from hair. A second treatment must be done in 7 to 10 days to kill any newly hatched lice.''

(3) *For products formulated for use as a shampoo.* ''Apply to affected area until all the hair is thoroughly wet with product. Allow product to remain on area for 10 minutes but no longer. Add sufficient warm water to form a lather and shampoo as usual. Rinse thoroughly. A fine-toothed comb or a special lice/nit removing comb may be used to help remove dead lice or their eggs (nits) from hair. A second treatment must be done in 7 to 10 days to kill any newly hatched lice.''

(e) *Other required statements.*

(1) ''*Head Lice:* Head lice live on the scalp and lay small white eggs (nits) on the hair shaft close to the scalp. The nits are most easily found on the nape of the neck or behind the ears. All personal headgear, scarfs, coats, and bed linen should be disinfected by machine washing in hot water and drying, using the hot cycle of a dryer for at least 20 minutes. Personal articles of clothing or bedding that cannot be washed may be dry-cleaned, sealed in a plastic bag for a period of about 2 weeks, or sprayed with a product specifically designed for this purpose. Personal combs and brushes may be disinfected by soaking in hot water (above 130 ° F) for 5 to 10 minutes. Thorough vacuuming of rooms inhabited by infected patients is recommended.''

(2) ''*Pubic (Crab) Lice:* Pubic lice may be transmitted by sexual contact; therefore, sexual partners should be treated simultaneously to avoid reinfestation. The lice are very small and look almost like brown or grey dots on the skin. Pubic lice usually cause intense itching and lay small white eggs (nits) on the hair shaft generally close to the skin surface. In hairy individuals, pubic lice may be present on the short hairs of the thighs and trunk, underarms, and occasionally on the beard and mustache. Underwear should be disinfected by machine washing in hot water; then drying, using the hot cycle for at least 20 minutes.''

(3) ''*Body Lice:* Body lice and their eggs are generally found in the seams of clothing, particularly in the waistline and armpit area. They move to the skin to feed, then return to the seams of the clothing where they lay their eggs. Clothing worn and not laundered before treatment should be disinfected by the same procedure as described for head lice, except that sealing clothing in a plastic bag is not recommended for body lice because the nits (eggs) from these lice can remain dormant for a period of up to 30 days.''

Subpart H—Drug Products for the Control of Dandruff, Seborrheic Dermatitis, and Psoriasis

SOURCE: 56 FR 63568, Dec. 4, 1991, unless otherwise noted.

§ 358.701 Scope.

(a) An over-the-counter dandruff, seborrheic dermatitis, or psoriasis drug product in a form suitable for topical application is generally recognized as safe and effective and is not misbranded if it meets each of the conditions in this subpart and each general condition established in § 330.1 of this chapter.

(b) References in this subpart to regulatory sections of the Code of Federal Regulations are to chapter I of title 21 unless otherwise noted.

§ 358.703 Definitions.

As used in this subpart:

(a) *Coal tar.* The tar used for medicinal purposes that is obtained as a byproduct during the destructive distillation of bituminous coal at temperatures in the range of 900 °C to 1,100 °C. It may be further processed using either extraction with alcohol and suitable dispersing agents and maceration times or fractional distillation with or without the use of suitable organic solvents.

(b) *Dandruff.* A condition involving an increased rate of shedding of dead epidermal cells of the scalp.

(c) *Psoriasis.* A condition of the scalp or body characterized by irritation,

itching, redness, and extreme excess shedding of dead epidermal cells.

(d) *Seborrheic dermatitis.* A condition of the scalp or body characterized by irritation, itching, redness, and excess shedding of dead epidermal cells.

(e) Selenium sulfide, micronized. Selenium sulfide that has been finely ground and that has a median particle size of approximately 5 micrometers (μm), with not more than 0.1 percent of the particles greater than 15 μm and not more than 0.1 percent of the particles less than 0.5 μm.

[56 FR 63568, Dec. 4, 1991, as amended at 59 FR 4001, Jan. 28, 1994]

§ 358.710 Active ingredients for the control of dandruff, seborrheic dermatitis, or psoriasis.

The active ingredient of the product consists of any of the following within the specified concentration established for each ingredient:

(a) *Active ingredients for the control of dandruff.* (1) Coal tar, 0.5 to 5 percent. When a coal tar solution, derivative, or fraction is used as the source of the coal tar, the labeling shall specify the identity and concentration of the coal tar source used and the concentration of the coal tar present in the final product.

(2) Pyrithione zinc, 0.3 to 2 percent when formulated to be applied and then washed off after brief exposure.

(3) Pyrithione zinc, 0.1 to 0.25 percent when formulated to be applied and left on the skin or scalp.

(4) Salicylic acid, 1.8 to 3 percent.

(5) Selenium sulfide, 1 percent.

(6) Selenium sulfide, micronized, 0.6 percent.

(7) Sulfur, 2 to 5 percent.

(b) *Active ingredients for the control of seborrheic dermatitis.* (1) Coal tar, 0.5 to 5 percent. When a coal tar solution, derivative, or fraction is used as the source of the coal tar, the labeling shall specify the identity and concentration of the coal tar source used and the concentration of the coal tar present in the final product.

(2) Pyrithione zinc, 0.95 to 2 percent when formulated to be applied and then washed off after brief exposure.

(3) Pyrithione zinc, 0.1 to 0.25 percent when formulated to be applied and left on the skin or scalp.

(4) Salicylic acid, 1.8 to 3 percent.

(5) Selenium sulfide, 1 percent.

(c) *Active ingredients for the control of psoriasis.* (1) Coal tar, 0.5 to 5 percent. When a coal tar solution, derivative, or fraction is used as the source of the coal tar, the labeling shall specify the identity and concentration of the coal tar source used and the concentration of the coal tar present in the final product.

(2) Salicylic acid, 1.8 to 3 percent.

[56 FR 63568, Dec. 4, 1991, as amended at 59 FR 4001, Jan. 28, 1994]

§ 358.720 Permitted combinations of active ingredients.

Salicylic acid identified in § 358.710(a)(4) may be combined with sulfur identified in § 358.710(a)(6) provided each ingredient is present within the established concentration and the product is labeled for the control of dandruff.

§ 358.750 Labeling of drug products for the control of dandruff, seborrheic dermatitis, or psoriasis.

(a) *Statement of identity.* The labeling of the product contains the established name of the drug, if any, and identifies the product with one or more of the following, as appropriate:

(1) ''Dandruff (insert product form)'' or ''antidandruff (insert product form)''.

(2) ''Seborrheic dermatitis (insert product form)''.

(3) ''Psoriasis (insert product form)''.

(b) *Indications.* The labeling of the product states, under the heading ''Indications,'' the phrase listed in paragraph (b)(1) of this section and may contain any of the terms listed in paragraph (b)(2) or (b)(3) of this section. Other truthful and nonmisleading statements, describing only the indications for use that have been established and listed in paragraph (b) of this section, may also be used, as provided in § 330.1(c)(2) of this chapter, subject to the provisions of section 502 of the Federal Food, Drug, and Cosmetic Act (the act) relating to misbranding and the prohibition in section 301(d) of the act against the introduction or delivery for introduction into interstate commerce of unapproved new drugs in violation of section 505(a) of the act.

(1) (''For relief of'' or ''Controls'') ''the symptoms of'' (select one or more of the following, as appropriate: ''dandruff,'' ''seborrheic dermatitis,'' and/or ''psoriasis.'')

(2) The following terms or phrases may be used in place of or in addition to the words ''For the relief of'' or ''Controls'' in the indications in paragraph (b)(1) of this section: ''fights,'' ''reduces,'' ''helps eliminate,'' ''helps stop,'' ''controls recurrence of,'' ''fights recurrence of,'' ''helps prevent recurrence of,'' ''reduces recurrence of,'' ''helps eliminate recurrence of,'' ''helps stop recurrence of.''

(3) The following terms may be used in place of the words ''the symptoms of'' in the indications in paragraph (b)(1) of this section: (''skin'' and/or ''scalp,'' as appropriate) (select one or more of the following: ''itching,'' ''irritation,'' ''redness,'' ''flaking,'' ''scaling,'') ''associated with.''

(c) *Warnings.* The labeling of the product contains the following warnings under the heading ''Warnings'':

(1) *For products containing any ingredient identified in §358.710.* (i) ''For external use only.''

(ii) ''Avoid contact with the eyes. If contact occurs, rinse eyes thoroughly with water.''

(iii) ''If condition worsens or does not improve after regular use of this product as directed, consult a doctor.''

(2) *For any product containing coal tar identified in §358.710(a), (b), or (c).* (i) ''Use caution in exposing skin to sunlight after applying this product. It may increase your tendency to sunburn for up to 24 hours after application.''

(ii) ''Do not use for prolonged periods without consulting a doctor.''

(3) *For products containing coal tar when formulated to be applied and left on the skin (e.g., creams, ointments, lotions).* ''Do not use this product in or around the rectum or in the genital area or groin except on the advice of a doctor.''

(4) *For products containing coal tar identified in §358.710(c) for the control of psoriasis.* ''Do not use this product with other forms of psoriasis therapy such as ultraviolet radiation or prescription drugs unless directed to do so by a doctor.''

(5) *For products containing any ingredient identified in §358.710(b) or (c) for the control of seborrheic dermatitis or psoriasis.* ''If condition covers a large area of the body, consult your doctor before using this product.''

(d) *Directions.* The labeling of the product contains the following information under the heading ''Directions.'' More detailed directions applicable to a particular product formulation may also be included.

(1) *For products containing active ingredients for the control of dandruff, seborrheic dermatitis, or psoriasis when formulated to be applied and then washed off after brief (a few minutes) exposure (e.g, shampoos, preshampoo rinses, postshampoo rinses).* ''For best results use at least twice a week or as directed by a doctor.''

(2) *For products containing active ingredients for the control of dandruff, seborrheic dermatitis, or psoriasis when formulated so as to be applied and left on the skin or scalp (e.g., creams, ointments, lotions, hairgrooms).* ''Apply to affected areas one to four times daily or as directed by a doctor.''

(3) *For products containing active ingredients for the control of seborrheic dermatitis or psoriasis of the skin when formulated as soaps.* ''Use on affected areas in place of your regular soap.''

(e) The word ''physician'' may be substituted for the word ''doctor'' in any of the labeling statements in this section.

PART 361—PRESCRIPTION DRUGS FOR HUMAN USE GENERALLY RECOGNIZED AS SAFE AND EFFECTIVE AND NOT MISBRANDED: DRUGS USED IN RESEARCH

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 371; 42 U.S.C. 262.

§361.1 Radioactive drugs for certain research uses.

(a) Radioactive drugs (as defined in §310.3(n) of this chapter) are generally recognized as safe and effective when administered, under the conditions set forth in paragraph (b) of this section, to human research subjects during the course of a research project intended to obtain basic information regarding the

metabolism (including kinetics, distribution, and localization) of a radioactively labeled drug or regarding human physiology, pathophysiology, or biochemistry, but not intended for immediate therapeutic, diagnostic, or similar purposes or to determine the safety and effectiveness of the drug in humans for such purposes (i.e., to carry out a clinical trial). Certain basic research studies, e.g., studies to determine whether a drug localizes in a particular organ or fluid space and to describe the kinetics of that localization, may have eventual therapeutic or diagnostic implications, but the initial studies are considered to be basic research within the meaning of this section.

(b) The conditions under which use of radioactive drugs for research are considered safe and effective are:

(1) *Approval by Radioactive Drug Research Committee.* A Radioactive Drug Research Committee, composed and approved by the Food and Drug Administration in accordance with paragraph (c) of this section, has determined, in accordance with the standards set forth in paragraph (d) of this section, that:

(i) The pharmacological dose is within the limits set forth in paragraph (b)(2) of this section;

(ii) The radiation dose is within the limits set forth in paragraph (b)(3) of this section;

(iii) The radiation exposure is justified by the quality of the study being undertaken and the importance of the information it seeks to obtain;

(iv) The study meets the other requirements set forth in paragraph (d) of this section regarding qualifications of the investigator, proper licensure for handling radioactive materials, selection and consent of research subjects, quality of radioactive drugs used, research protocol design, reporting of adverse reactions, and approval by an appropriate Institutional Review Committee; and

(v) The use of the radioactive drug in human subjects has the approval of the Radioactive Drug Research Committee.

(2) *Limit on pharmacological dose.* The amount of active ingredient or combination of active ingredients to be administered shall be known not to cause any clinically detectable pharmacological effect in human beings. If the same active ingredients (exclusive of the radionuclide) are to be administered simultaneously, e.g., under a ''Investigational New Drug Application'' or for a therapeutic use in accordance with labeling for a drug approved under part 314 of this chapter, the total amount of active ingredients including the radionuclide shall be known not to exceed the dose limitations applicable to the separate administration of the active ingredients excluding the radionuclide.

(3) *Limit on radiation dose.* The amount of radioactive material to be administered shall be such that the subject receives the smallest radiation dose with which it is practical to perform the study without jeopardizing the benefits to be obtained from the study.

(i) Under no circumstances may the radiation dose to an adult research subject from a single study or cumulatively from a number of studies conducted within 1 year be generally recognized as safe if such dose exceeds the following:

Whole body, active blood-forming organs, lens of the eye, and gonads:

	Rems
Single dose	3
Annual and total dose commitment	5
Other organs:	
Single dose	5
Annual and total dose commitment	15

(ii) For a research subject under 18 years of age at his last birthday, the radiation dose shall not exceed 10 percent of that set forth in paragraph (b)(3)(i) of this section.

(iii) All radioactive material included in the drug either as essential material or as a significant contaminant or impurity shall be included when determining the total radiation doses and dose commitments. Radiation doses from x-ray procedures that are part of the research study (i.e., would not have occurred but for the study) shall also be included. The possibility of followup studies shall be considered for inclusion in the dose calculations.

(iv) Numerical definitions of dose shall be based on an absorbed fraction

method of radiation absorbed dose calculation, such as the system set forth by the Medical Internal Radiation Dose Committee of the Society of Nuclear Medicine, or the system set forth by the International Commission on Radiological Protection.

(c) A Radioactive Drug Research Committee, in order to comply with paragraph (b)(1) of this section, shall be composed, shall function, and shall obtain and maintain approval of the Food and Drug Administration in conformity with the following:

(1) *Membership.* A Radioactive Drug Research Committee shall consist of at least five individuals. Each committee shall include the following three individuals: (i) A physician recognized as a specialist in nuclear medicine, (ii) a person qualified by training and experience to formulate radioactive drugs, and (iii) a person with special competence in radiation safety and radiation dosimetry. The remainder of the committee shall consist of individuals qualified in various disciplines pertinent to the field of nuclear medicine (e.g., radiology, internal medicine, clinical pathology, hematology, endocrinology, radiation therapy, radiation physics, radiation biophysics, health physics, and radiopharmacy). Membership shall be sufficiently diverse to permit expert review of the technical and scientific aspects of proposals submitted to the committee. The addition of consultants in other pertinent medical disciplines is encouraged. A Radioactive Drug Research Committee shall be either associated with a medical institution operated for care of patients and with sufficient scientific expertise to allow for selection of committee members from its faculty, or with a committee established by a State authority to provide advice on radiation health matters. Joint committees involving more than one medical institution which have been established in order to achieve a high level and diversity of experience will be acceptable. The Director of the Center for Drug Evaluation and Research may modify any of the foregoing requirements in a particular situation where alternative factors provide substantially the same composition and association.

(2) *Function.* Each Radioactive Drug Research Committee shall select a chairman, who shall sign all applications, minutes, and reports of the committee. Each committee shall meet at least once each quarter in which research activity has been authorized or conducted. A quorum consisting of more than 50 percent of the membership must be present with appropriate representation of the required fields of specialization. Minutes shall be kept and shall include the numerical results of votes on protocols involving use in human subjects. No member shall vote on a protocol in which he is an investigator.

(3) *Reports.* Each Radioactive Drug Research Committee shall submit an annual report on or before January 31 of each year to the Food and Drug Administration, Center for Drug Evaluation and Research, HFD–160, 5600 Fishers Lane, Rockville, MD 20857. The annual report shall include the names and qualifications of the members of, and of any consultants used by, the Radioactive Drug Research Committee, and, for each study conducted during the preceding year, a summary of information presented in the following format:

REPORT ON RESEARCH USE OF RADIOACTIVE DRUG

1. Title of the research project.
2. Brief description of the purpose of the research project.
3. Name of the investigator responsible.
4. Pharmacological dose:
a. Active ingredients.
b. Maximum amount administered per subject.
5. Name of the radionuclide(s) used, including any present, as significant contaminants or impurities.
6. Radiation absorbed dose. Provide the maximum dose commitement to the whole body and each organ specified in 21 CFR 361.1(b)(3)(i) that was received by a representative subject and the calculations or references that were used to estimate these maximum dose commitments. The report shall include the dose contribution of both the administered radionuclide(s) and any X-ray procedures associated with the study. If the study elicits data on the uptake or excretion of the radioactive drug pertinent to the estimation of dose commitment, report the mean value and range of values. For each subject provide:
(a) Age, sex, and approximate weight.

(b) Total activity of each radionuclide administered for each radioactive drug used in the study. Report each X-ray procedure used in conjunction with the study.

(c) If the subject has participated in other radioactive drug research studies, report the name of the radioactive drug used in these other studies, the date of administration, and the total activity of each radionuclide administered. If any X-ray procedures were used, identify the X-ray procedure(s) and include an estimate of the absorbed radiation doses.

(d) If more than one administration of a radioactive drug per subject, cumulative radiation dose and dose commitment, expressed as whole body, active blood-forming organs, lens of the eye, gonads, and other organ doses from the administered radionuclides.

7. A claim of confidentiality, if any.

NOTE: Contents of this report are available for public disclosure unless confidentiality is requested by the investigator and it is adequately shown by the investigator that the report constitutes a trade secret or confidential commercial information as defined in 21 CFR 20.61.

_____
Investigator

_____
Chairman, Radioactive Drug Research Committee

At any time a proposal is approved which involves exposure either of more than 30 research subjects, or of any research subject under 18 years of age, the committee shall immediately submit to the Food and Drug Administration a special summary of information in the format shown in this paragraph. Contents of these reports are available for public disclosure, unless confidentiality is requested by the investigator and it is adequately shown by the investigator that the report constitutes a trade secret or confidential commercial information as defined in § 20.61 of this chapter.

(4) *Approval.* Each Radioactive Drug Research Committee shall be specifically approved by the Center for Drug Evaluation and Research of the Food and Drug Administration. Applications shall be submitted to the Food and Drug Administration, Center for Drug Evaluation and Research, HFD–160, 5600 Fishers Lane, Rockville, MD 20857, and shall contain the names and qualifications of the members of the committee, and a statement that the committee agrees to comply with the requirements set forth in this section. Approval shall be based upon an assessment of the qualifications of the members of the committee, and the assurance that all necessary fields of expertise are covered. Approval of a committee may be withdrawn at any time for failure of the committee to comply with any of the requirements of this section. Approval of a committee shall remain effective unless and until the FDA withdraws such approval. Changes in membership and applications for new members shall be submitted to the Food and Drug Administration as soon as, or before, vacancies occur on the committee.

(5) *Monitoring.* The Food and Drug Administration shall conduct periodic reviews of approved committees. Monitoring of the activities of the committee shall be conducted through review of its annual report, through review of minutes and full protocols for certain studies, and through on-site inspections.

(d) In making the determination required in paragraph (b)(1) of this section, a Radioactive Drug Research Committee shall consider the following requirements and assure that each is met:

(1) *Radiation dose to subjects.* To assure that the radiation dose to research subjects is as low as practicable to perform the study and meet the criteria of § 361.1(b)(3), the Radioactive Drug Research Committee shall require that:

(i) The investigator provide absorbed dose calculations based on biologic distribution data available from published literature or from other valid studies.

(ii) The investigator provide for an acceptable method of radioassay of the radioactive drug prior to its use to assure that the dose calculations actually reflect the administered dose.

(iii) The radioactive drug chosen for the study has that combination of half-life, types of radiations, radiation energy, metabolism, chemical properties, etc., which results in the lowest dose to the whole body or specific organs with which it is possible to obtain the necessary information.

(iv) The investigator utilize adequate and appropriate instrumentation for the detection and measurement of the specific radionuclide.

(2) *Pharmacological dosage.* To determine that the amount of active ingredients to be administered does not exceed the limitations set forth in paragraph (b)(2) of this section, the committee shall require that the investigator provide pharmacological dose calculations based on data available from published literature or from other valid human studies.

(3) *Qualifications of investigators.* Each investigator shall be qualified by training and experience to conduct the proposed research studies.

(4) *License to handle radioactive materials.* The responsible investigator or institutions shall, in the case of reactor-produced isotopes, be licensed by the Nuclear Regulatory Commission or Agreement State to possess and use the specific radionuclides for research use or be a listed investigator under a broad license, or in the case of non-reactor-produced isotopes, be licensed by other appropriate State or local authorities, when required by State or local law, to possess and use the specific radionuclides for research use.

(5) *Human research subjects.* Each investigator shall select appropriate human subjects and shall obtain the review and approval of an institutional review committee that conforms to the requirements of part 56 of this chapter, and shall obtain the consent of the subjects or their legal representatives in accordance with part 50 of this chapter. The research subjects shall be at least 18 years of age and legally competent. Exceptions are permitted only in those special situations when it can be demonstrated to the committee that the study presents a unique opportunity to gain information not currently available, requires the use of research subjects less than 18 years of age, and is without significant risk to the subject. Studies involving minors shall be supported with review by qualified pediatric consultants to the Radioactive Drug Research Committee. Each female research subject of childbearing potential shall state in writing that she is not pregnant, or, on the basis of a pregnancy test be confirmed as not pregnant, before she may participate in any study.

(6) *Quality of radioactive drug.* The radioactive drug used in the research study shall meet appropriate chemical, pharmaceutical, radiochemical, and radionuclidic standards of identity, strength, quality, and purity as needed for safety and be of such uniform and reproducible quality as to give significance to the research study conducted. The Radioactive Drug Research Committee shall determine that radioactive materials for parenteral use are prepared in sterile and pyrogen-free form.

(7) *Research protocol.* No matter how small the amount of radioactivity, no study involving administration of a radioactive drug, as defined in § 310.3(n) of this chapter, to research subjects under this section, shall be permitted unless the Radioactive Drug Research Committee concludes, in its judgment, that scientific knowledge and benefit is likely to result from that study. Therefore, the protocol shall be based upon a sound rationale derived from appropriate animal studies or published literature and shall be of sound design such that information of scientific value may result. The radiation dose shall be both sufficient and no greater than necessary to obtain valid measurement. The projected number of subjects shall be sufficient but no greater than necessary for the purpose of the study. The number of subjects shall also reflect the fact that the study is intended to obtain basic research information referred to in paragraph (a) of this section and not intended for immediate therapeutic, diagnostic or similar purposes or to determine the safety and effectiveness of the drug in humans for such purposes (i.e., to carry out a clinical trial).

(8) *Adverse reactions.* The investigator shall immediately report to the Radioactive Drug Research Committee all adverse effects associated with the use of the radioactive drug in the research study. All adverse reactions probably attributable to the use of the radioactive drug in the research study shall be immediately reported by the Radioactive Drug Research Committee to the Food and Drug Administration, Center for Drug Evaluation and Research, HFD–160, 5600 Fishers Lane, Rockville, MD 20857.

(9) *Approval by an institutional review board.* The investigator shall obtain the

review and approval of an institutional review board that conforms to the requirements of part 56 of this chapter.

(e) The results of any research conducted pursuant to this section as part of the evaluation of a drug pursuant to part 312 of this chapter shall be included in the submissions required under part 312 of this chapter.

(f) A radioactive drug prepared, packaged, distributed, and primarily intended for use in accordance with the requirements of this section shall be exempt from section 502(f)(1) of the act and §§ 201.5 and 201.100 of this chapter if the packaging, label, and labeling are in compliance with Federal, State, and local law regarding radioactive materials and if the label of the immediate container and shielded container, if any, either separate from or as part of any label and labeling required for radioactive materials by the Nuclear Regulatory Commission or by State or local radiological health authorities bear the following:

(1) The statement ''Caution: Federal law prohibits dispensing without prescription'';

(2) The statement ''To be administered in compliance with the requirements of Federal regulations regarding radioactive drugs for research use (21 CFR 361.1)'';

(3) The established name of the drug, if any;

(4) The established name and quantity of each active ingredient;

(5) The name and half-life of the radionuclide, total quantity of radioactivity in the drug product's immediate container, and amount of radioactivity per unit volume or unit mass at a designated referenced time;

(6) The route of administration, if it is for the other than oral use;

(7) The net quantity of contents;

(8) An identifying lot or control number from which it is possible to determine the complete manufacturing history of the package of the drug;

(9) The name and address of the manufacturer, packer, or distributor;

(10) The expiration date, if any;

(11) If the drug is intended for parenteral use, a statement as to whether the contents are sterile;

(12) If the drug is for other than oral use, the names of all inactive ingredients, except that:

(i) Trace amounts of harmless substances added solely for individual product identification need not be named.

(ii) If the drug is intended for parenteral use, the quantity or proportion of all inactive ingredients, except that ingredients added to adjust pH or to make the drug isotonic may be declared by name and a statement of their effect; if the vehicle is water for injection, it need not be named. *Provided, however,* That in the case of containers too small or otherwise unable to accommodate a label with sufficient space to bear all such information, the information required by paragraphs (f)(1) and (12) of this section may be placed on the shielded container only.

[40 FR 31308, July 25, 1975, as amended at 40 FR 44543, Sept. 29, 1975; 42 FR 15674, Mar. 22, 1977; 43 FR 14646, Apr. 7, 1978; 46 FR 8955, Jan. 27, 1981; 49 FR 44460, Nov. 7, 1984; 50 FR 8996, Mar. 6, 1985; 55 FR 11582, Mar. 29, 1990; 56 FR 10806, Mar. 14, 1991]

PART 369—INTERPRETATIVE STATEMENTS RE WARNINGS ON DRUGS AND DEVICES FOR OVER-THE-COUNTER SALE

Subpart A—Definitions and Interpretations

Sec.
369.1 Purpose of issuance.
369.2 Definitions.
369.3 Warnings required on drugs exempted from prescription-dispensing requirements of section 503(b)(1)(C).
369.4 Warnings suggested for drugs by formal or informal statements of policy.
369.5 Warnings required on insulin intended for over-the-counter sale.
369.6 [Reserved]
369.7 Warnings required by official compendia.
369.8 Warning statements in relation to conditions for use.
369.9 General warnings re accidental ingestion by children.
369.10 Conspicuousness of warning statements.

Subpart B—Warning and Caution Statements for Drugs

369.20 Drugs; recommended warning and caution statements.

369.21 Drugs; warning and caution statements required by regulations.
369.22 Drugs; warning and caution statements specifically required by law.

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 371.

SOURCE: 39 FR 11745, Mar. 29, 1974, unless otherwise noted.

Subpart A—Definitions and Interpretations

§369.1 Purpose of issuance.

The warning and caution statements suggested in subparts B and C of this part, for inclusion in the label or labeling of drugs and devices subject to section 502(d) and (f)(2) and other relevant provisions of the Federal Food, Drug, and Cosmetic Act are issued for the purpose of assisting industry in preparing proper labeling for these articles for over-the-counter sale and in meeting the legal requirements of the act that the label or labeling of drugs and devices bear adequate warnings, in such manner and form as are necessary for the protection of users. Only section 502(d) of the act requires use of the specific language included in these suggested warning and caution statements. These suggested warning or caution statements are illustrative of those that may be necessary or desirable. It is the responsibility of the manufacturer, packer, shipper, or distributor in interstate commerce to see that such statements are adequate for compliance with the provisions of the law. Omission of any article from this suggested list does not relieve drugs and devices subject to provisions of the act from bearing adequate warning or caution statements where such statements are necessary or desirable for the protection of the user.

§369.2 Definitions.

(a) As used in this part, the term *act* means the Federal Food, Drug, and Cosmetic Act.

(b) The terms *drugs* and *devices* are defined in section 201(g) and (k) of the act.

(c) Official compendia are defined in section 201(j) of the act.

§369.3 Warnings required on drugs exempted from prescription-dispensing requirements of section 503(b)(1)(C).

Drugs exempted from prescription-dispensing requirements under section 503(b)(1)(C) of the act are subject to the labeling requirements prescribed in §310.201(a) of this chapter. Although, for convenience, warning and caution statements for a number of the drugs named in §310.201 of this chapter (cross-referenced in the text of this part) are included in subpart B of this part, the inclusion of such drugs in §§369.20, 369.21, 369.22 in no way affects the requirements for compliance with §310.201(a) of this chapter, or the provisions of an effective application pursuant to section 505(b) of the act.

§369.4 Warnings suggested for drugs by formal or informal statements of policy.

The warning and caution statements included in subpart B of this part in no way affect any warning statement suggested for such drugs or devices by any statement of policy or interpretation in subchapter C of this chapter.

[39 FR 11745, Mar. 29, 1974, as amended at 40 FR 13496, Mar. 27, 1975]

§369.5 Warnings required on insulin intended for over-the-counter sale.

Warning and caution statements for insulin products sold over the counter must comply with the specific labeling provisions of the act and §429.11 of this chapter.

§369.6 [Reserved]

§369.7 Warnings required by official compendia.

Any drug included in the official compendia defined by the act shall bear such warning or caution statement as may be required by such compendia, and no statement in subpart B or subpart C of this part is intended to alter, modify, or permit the omission of any such statement required by such compendia.

§369.8 Warning statements in relation to conditions for use.

The mention in any warning or caution statement included in subparts A,

B, and C of this part, of a disease condition does not imply a finding on the part of the Food and Drug Administration that any drug or device is efficacious in such condition; nor is any drug or device bearing labeling referring to such disease condition precluded from regulatory action under the applicable provisions of the act if such claim is considered to be misbranding.

§ 369.9 General warnings re accidental ingestion by children.

Section 369.20 includes under certain items, but not all medicines, the statement: ''Warning—Keep this and all medicines out of children's reach. In case of accidental overdose, contact a physician immediately'', or ''Warning—Keep out of the reach of children''. However, in view of the possibility of accidental ingestion of drugs, it is not only suggested but is recommended that one of these statements be used on the label of all drug products.

§ 369.10 Conspicuousness of warning statements.

Necessary warning statements should appear in the labeling prominently and conspicuously as compared to other words, statements, designs, and devices, and in bold type on clearly contrasting background, in order to comply with the provisions of section 502(c) and (f)(2) of the act. The warning statements should be placed in the labeling in juxtaposition with the directions for use and, in any case, should appear on the label when there is sufficient label space in addition to mandatory label information.

Subpart B—Warning and Caution Statements for Drugs

§ 369.20 Drugs; recommended warning and caution statements.

ACETANILID.

Warning—Do not exceed recommended dosage. Overdosage or continued use may result in serious blood disturbances.

ACETOPHENETIDIN CONTAINING PREPARATIONS. (See § 201.309 of this chapter.)

Warning—This medication may damage the kidneys when used in large amounts or for a long period of time. Do not take more than the recommended dosage, nor take regularly for longer than 10 days without consulting your physician.

ANESTHETICS FOR EXTERNAL USE (LOCAL ANESTHETICS). (See also § 310.201(a)(19) and (23) of this chapter.)

Caution—Do not use in the eyes. Not for prolonged use. If the condition for which this preparation is used persists or if a rash or irritation develops, discontinue use and consult physician.

ANTIHISTAMINICS FOR EXTERNAL USE (EXCEPT PREPARATIONS FOR OPHTHALMIC USE).

Caution—Do not use in the eyes. If the condition for which this preparation is used persists or if a rash or irritation develops, discontinue use and consult physician.

ANTIHISTAMINICS, ORAL. (See also § 310.201(a)(4) and (a)(24) of this chapter.)

Caution—This preparation may cause drowsiness. Do not drive or operate machinery while taking this medication. Do not give to children under 6 years of age or exceed the recommended dosage unless directed by physician.

The reference to drowsiness is not required on preparations for the promotion of sleep or on preparations that are shown not to produce drowsiness.

ANTIPERSPIRANTS.

Do not apply to broken skin. If a rash develops, discontinue use.

ANTIPYRINE.

Warning—Do not exceed recommended dosage. If skin rash appears, discontinue use and consult physician.

ANTISEPTICS FOR EXTERNAL USE.

Caution—In case of deep or puncture wounds or serious burns, consult physician. If redness, irritation, swelling, or pain persists or increases or if infection occurs discontinue use and consult physician.

The reference to wounds and burns is not required on preparations intended solely for diaper rash.

ARSENIC PREPARATIONS.

Warning—Frequent or prolonged use may cause serious injury. Do not exceed recommended dosage. Keep out of the reach of children.

BELLADONNA PREPARATIONS AND PREPARATIONS OF ITS ALKALOIDS (ATROPINE, HYOSCYAMINE, AND SCOPOLAMINE (HYOSCINE); HYOSCYAMUS, STRAMONIUM, THEIR DERIVATIVES, AND RELATED DRUG PREPARATIONS.

Warning—Not to be used by persons having glaucoma or excessive pressure within the eye, by elderly persons (where undiagnosed glaucoma or excessive pressure within the eye occurs most frequently), or by children under 6 years of age, unless directed by a physician. Discontinue use if blurring of vision, rapid pulse, or dizziness occurs. Do not exceed recommended dosage. Not for frequent or prolonged use. If dryness of the mouth occurs, decrease dosage. If eye pain occurs, discontinue use and see your physician immediately as this may indicate undiagnosed glaucoma.

In the case of scopolamine or scopolamine aminoxide preparations indicated for insomnia, the portion of the above warning that reads ''children under 6 years of age'' should read instead ''children under 12 years of age''.

BORIC ACID (POWDERED, CRYSTALLINE, OR GRANULAR).

Warning—Do not use as a dusting powder, especially on infants, or take internally. Use only as a solution. Do not apply to badly broken or raw skin, or to large areas of the body.

BROMIDES.

Caution—Use only as directed. Do not give to children or use in the presence of kidney disease. If skin rash appears or if nervous symptoms persist, recur frequently, or are unusual, discontinue use and consult physician.

CARBOLIC ACID (PHENOL) PREPARATIONS (MORE THAN 0.5 PERCENT) FOR EXTERNAL USE.

Warning—Use according to directions. Do not apply to large areas of the body. If applied to fingers or toes, do not bandage.

CATHARTICS AND LAXATIVES—IRRITANTS AND OTHER PERISTALTIC STIMULANTS.

Warning—Do not use when abdominal pain, nausea, or vomiting are present. Frequent or prolonged use of this preparation may result in dependence on laxatives.

Mercury preparations should have added to the ''frequent use'' statement, the words ''and serious mercury poisoning''.

Phenolphthalein preparations should bear, in addition to the general warning, the following statement:

Caution—If skin rash appears, do not use this or any other preparation containing phenolphthalein.

See also Mineral Oil Laxatives.

CHLORATES: MOUTH WASH OR GARGLE.

Avoid swallowing.

COBALT PREPARATIONS (See also § 250.106 of this chapter.)

Warning—Do not exceed the recommended dosage. Do not administer to children under 12 years of age unless directed by physician. Do not use for more than 2 months unless directed by physician.

This warning is not required on articles containing not more than 0.5 milligram of cobalt as a cobalt salt per dosage unit and which recommend administration of not more than 0.5 milligram per dose and not more than 2 milligrams per 24-hour period.

''COUGH-DUE-TO-COLD'' PREPARATIONS. (See also § 310.201(a)(20) of this chapter.)

Warning—Persons with a high fever or persistent cough should not use this preparation unless directed by physician.

COUNTERIRRITANTS AND RUBEFACIENTS.

Caution—Do not apply to irritated skin or if excessive irritation develops. Avoid getting into the eyes or on mucous membranes.

If offered for use in arthritis or rheumatism, in juxtaposition therewith, the statement:

Caution—If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age consult a physician immediately.

See also ''Salicylates'' in this section for additional warnings for preparations containing methyl salicylate.

CREOSOTE, CRESOLS, GUAIACOL, AND SIMILAR SUBSTANCES IN PREPARATIONS FOR EXTERNAL USE.

Caution—Do not apply to large areas of the body.

CREOSOTE, CRESOLS, GUAIACOL, AND SIMILAR SUBSTANCES IN DOUCHE PREPARATIONS.

Warning—The use of solutions stronger than those recommended may result in severe local irritation, burns, or serious poisoning. Mix as directed before pouring into douche bag. Do not use more often than twice weekly unless directed by physician.

DENTURE RELINERS, PADS, AND CUSHIONS.

Warning—For temporary use only. Long-term use of this product may lead to faster bone loss, continuing irritation, sores, and tumors. For Use Only Until a Dentist Can Be Seen.

DENTURE REPAIR KITS.

Warning—For emergency repairs only. Long-term use of home-repaired dentures may cause faster bone loss, continuing irritation, sores, and tumors. This kit for emergency use only. See Dentist Without Delay.

DIARRHEA PREPARATIONS.

Warning—Do not use for more than 2 days or in the presence of high fever or in infants or children under 3 years of age unless directed by a physician.

DOUCHE PREPARATIONS.

Warning—Do not use more often than twice weekly unless directed by physician.

See also Creosote * * * Douche for additional warning.

DRESSINGS, PROTECTIVE SPRAY-ON TYPE. (See also § 310.201(a) (11) and (18) of this chapter.)

Warning—In case of deep or puncture wounds or serious burns consult physician. If redness, irritation, swelling or pain persists or increases or if infection occurs consult physician. Keep away from eyes or other mucous membranes. Avoid inhaling.

See also Dispensers Pressurized by Gaseous Propellants * * * for additional warnings to be included for products under pressure.

IODINE AND IODIDES (ORAL).

Caution—If a skin rash appears, discontinue use and consult physician.

MERCURY PREPARATIONS FOR EXTERNAL USE.

Warning—Discontinue use if rash or irritation develops or if condition for which used persists. Frequent or prolonged use, or application to large areas may cause serious mercury poisoning.

MINERAL OIL LAXATIVES. (See also § 201.302 of this chapter.)

Caution—Take only at bedtime. Avoid prolonged use. Do not administer to infants or young children, in pregnancy, or to bedridden or aged patients unless directed by physician.

NASAL PREPARATIONS: VASOCONSTRICTORS (PHENYLPROPANOLAMINE).

Caution—Do not exceed recommended dosage.

NUX VOMICA AND STRYCHNINE PREPARATIONS.

Warning—Do not exceed the recommended dosage. Keep out of the reach of children.

OPHTHALMIC PREPARATIONS. (See also § 200.50 of this chapter.)

Boric acid offered for use in the preparation of ophthalmic solutions should bear the statement: Prepare solution by boiling in water. Store in a sterile container. Prepare sufficient for one day's use and discard unused portion.

PHENACETIN-CONTAINING PREPARATION. (See acetophenetidin.)

PHENYLPROPANOLAMINE HYDROCHLORIDE PREPARATIONS, ORAL.

Caution—Individuals with high blood pressure, heart disease, diabetes, or thyroid disease should use only as directed by physician.

POTASSIUM PERMANGANATE AQUEOUS SOLUTIONS (CONTAINING NOT MORE THAN 0.04 PERCENT POTASSIUM PERMANGANATE). (See § 250.108 of this chapter.)

Warning—For external use on the skin only. Severe injury may result from use internally or as a douche. Avoid contact with mucous membranes.

QUININE AND OTHER CINCHONA DERIVATIVES (EXCEPT FOR USE IN MALARIA).

Caution—Discontinue use if ringing in the ears, deafness, skin rash, or visual disturbances occur.

RESINS, OLEORESINS, AND VOLATILE OILS.

Caution—If nausea, vomiting, abdominal discomfort, diarrhea, or skin rash occurs, discontinue use and consult physician.

RESORCINOL (NOT THE MONOACETATE) HAIR PREPARATIONS.

Caution—Excessive use of this preparation may temporarily discolor blond, white, or red hair.

SALICYLATES, INCLUDING ASPIRIN AND SALICYLAMIDE (EXCEPT METHYL SALICYLATE, EFFERVESCENT SALICYLATE PREPARATIONS, AND PREPARATIONS OF AMINOSALICYLIC ACID AND ITS SALTS). (See also § 201.314 of this chapter.)

Warning—Keep this and all medicines out of children's reach. In case of accidental overdose, contact a physician immediately; or

Warning—Keep out of the reach of children.

If the article is an aspirin preparation, it should bear the first of the above two warning statements. In either case, the above information should appear on the label.

Caution—For children under 3 years of age, consult your physician; or

Caution—For younger children, consult your physician.

One of the two immediately preceding caution statements is required on the label of all aspirin tablets, but such a statement is not required on the labels of other salicylates clearly offered for administration to adults only.

If offered for use in arthritis or rheumatism, in juxtaposition therewith, the statement:

Caution—If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age, consult a physician immediately.

SALICYLATES: METHYL SALICYLATE (WINTERGREEN OIL). See also §§ 201.303 and 201.314 of this chapter.

Warning—Do not use otherwise than as directed. Keep out of the reach of children to avoid accidental poisoning.

If the preparation is a counter-irritant or rubefacient the statement:

Caution—Discontinue use if excessive irritation of the skin develops. Avoid getting into the eyes or on mucous membranes.

If offered for use in arthritis or rheumatism, in juxtaposition therewith, the statement:

Caution—If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age consult a physician immediately.

SILVER.

Caution—Frequent or prolonged use of this preparation may result in permanent discoloration of skin and mucous membranes.

SODIUM PERBORATE MOUTHWASH AND GARGLE AND TOOTHPASTE.

Caution—Discontinue use if irritation or inflammation develops, or increases. Avoid swallowing.

SULFONAMIDE NOSE DROPS.

Caution—Do not use if a known allergy to sulfonamide drugs exists.

SULFUR PREPARATION FOR EXTERNAL USE.

Caution—If undue skin irritation develops or increases, discontinue use and consult physician.

THROAT PREPARATIONS FOR TEMPORARY RELIEF OF MINOR SORE THROAT: LOZENGES, TROCHES, WASHES, GARGLES, ETC. (See also § 201.315 of this chapter.)

Warning—Severe or persistent sore throat or sore throat accompanied by high fever, headache, nausea, and vomiting may be serious. Consult physician promptly. Do not use more than 2 days or administer to children under 3 years of age unless directed by physician.

TOOTHACHE PREPARATIONS.

For temporary use only until a dentist can be consulted.

ZINC STEARATE DUSTING POWDERS.

Warning—Keep out of the reach of infants and children; avoid inhaling.

[39 FR 11745, Mar. 29, 1974, as amended at 40 FR 8917, Mar. 3, 1975; 40 FR 13496, Mar. 27, 1975; 41 FR 10885, Mar. 15, 1976; 51 FR 27760, Aug. 1, 1986; 51 FR 35340, Oct. 2, 1986; 52 FR 15893, Apr. 30, 1987; 52 FR 30057, Aug. 12, 1987; 52 FR 47324, Dec. 11, 1987; 53 FR 7093, Mar. 4, 1988; 55 FR 31783, Aug. 3, 1990; 57 FR 58376, Dec. 9, 1992; 59 FR 43412, Aug. 23, 1994]

§ 369.21 Drugs; warning and caution statements required by regulations.

ACETAMINOPHEN (N-ACETYL-*p*-AMINOPHENOL) (See § 310.201(a)(1) of this chapter.)

Warning—Do not give to children under 3 years of age or use for more than 10 days unless directed by a physician.

If offered for use in arthritis, or rheumatism, in juxtaposition therewith, the statement:

Caution—If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age consult a physician immediately.

ALCOHOL RUBBING COMPOUND. (See 26 CFR 182.855(a)(5); The National Formulary, Tenth Edition 1955, pp. 27–28; and section 502(g) of the act).

Warning—For external use only. If taken internally serious gastric distrubances will result.

ANTIHISTAMINICS, ORAL (PHENYLTOLOXAMINE DIHYDROGEN CITRATE AND CHLOROTHEN CITRATE PREPARATIONS). (See § 310.201(a)(4) and (a)(24) of this chapter.)

Caution—This preparation may cause drowsiness. Do not drive or operate machinery while taking this medication. Do not give to children under 6 years of age or exceed the recommended dosage unless directed by physician.

If offered for symptoms of colds, the statement:

Caution—If relief does not occur within 3 days, discontinue use and consult physician.

CARBETAPENTANE CITRATE PREPARATIONS. (See Cough-Due-to-Cold Preparations.)

''COUGH-DUE-TO-COLD'' PREPARATIONS (CARBETAPENTANE CITRATE). (See § 310.201(a)(20) of this chapter.)

Warning—Keep out of the reach of children. Do not administer to children under 2 years of age unless directed by physician. Persistent cough may indicate the presence of a serious condition. Persons with a high fever or persistent cough should not use this preparation unless directed by physician.

DICYCLOMINE HYDROCHLORIDE WITH AN ANTACID. (See § 310.201(a)(8) of this chapter.)

Warning—Do not exceed the recommended dosage. Do not administer to children under 12 years of age or use for a prolonged period unless directed by physician, since persistent or recurring symptoms may indicate a serious disease requiring medical attention.

DIPHEMANIL METHYLSULFATE FOR EXTERNAL USE. (See § 310.201(a)(22) of this chapter.)

Caution—If redness, irritation, swelling, or pain persists or increases, discontinue use and consult physician.

DRUGS IN DISPENSERS PRESSURIZED BY GASEOUS PROPELLANTS. (See also § 310.201(a) (11) and (18) of this chapter.)

The warnings herein shall appear prominently and conspicuously, but in no case may the letters be less than 1/16 inch in height.

If the label of any package is too small to accommodate the warnings, the Commissioner may establish by regulation an acceptable alternative method, e.g., a type size smaller than 1/16 inch in height. A petition requesting such a regulation, as an amendment to this paragraph, shall be submitted to the Dockets Management Branch in the form established in part 10 of this chapter.

Warning—Avoid spraying in eyes. Contents under pressure. Do not puncture or incinerate. Do not store at temperature above 120° F. Keep out of reach of children.

In the case of products packaged in glass containers, the word ''break'' may be substituted for the word ''puncture.''

The words ''Avoid spraying in eyes'' may be deleted from the warning in the case of a product not expelled as a

spray, or that is intended to be used in the eyes.

In addition to the above warning, the label of a drug packaged in a self-pressurized container in which the propellant consists in whole or in part of a halocarbon or hydrocarbon shall bear the following warning:

Warning—Use only as directed. Intentional misuse by deliberately concentrating and inhaling the contents can be harmful or fatal.

The warning is not required for the following products:

(a) Products expelled in the form of a foam or cream, which contain less than ten percent propellant in the container;

(b) Products in a container with a physical barrier that prevents escape of the propellant at the time of use;

(c) Products of a net quantity of contents of less than 2 ozs. that are designed to release a measured amount of product with each valve actuation;

(d) Products of a net quantity of contents of less than ½ oz.

DYCLONINE HYDROCHLORIDE. (See § 310.201(a)(23) of this chapter.)

Caution—Do not use in the eyes. Not for prolonged use. Do not apply to large areas of the body. If redness, irritation, swelling, or pain persists or increases, discontinue use unless directed by physician. Do not use, but consult physician for deep or puncture wounds or serious burns. Do not use in case of rectal bleeding, as this may indicate serious disease.

HEXADENOL. (See § 310.201(a)(11) of this chapter.)

Caution—Do not use for treatment of serious burns or skin conditions or for conditions which persist for prolonged periods. In such cases, consult your physician. Do not spray in vicinity of eyes, mouth, nose, or ears. Do not store above 120° F.

INSULIN. (See § 429.11(c) of this chapter.)

Insulin (40 or 100 U.S.P. units per milliliter):

Caution—Do not remove stopper. Not for intravenous nor intramuscular use. Do not use after expiration date shown on outside wrapper or container. Do not use if drug has become viscous or if its color has become other than water clear.

In addition to the above warnings, the following statements should be included in the labeling: ''Keep in a cold place, avoid freezing. Failure to follow directions for use may lead to infection.'' Potamine zinc insulin, isophane insulin, lente insulin, semilente insulin, or ultralente insulin:

Caution—Do not remove stopper. Not for intravenous nor intramuscular use. Do not use after expiration date shown on outside wrapper or container. Do not substitute for any other insulin-containing drug unless directed by physician. Do not use when precipitate has become lumped or granular in appearance or has formed a deposit of solid particles on the wall of the container.

In addition to the above warnings for protamine zinc insulin * * *, the following statements should be included in the labeling of these preparations: ''Keep in a cold place, avoid freezing''; ''Shake carefully'' or ''Shake well before using'' or ''Shake well'' or ''Shake carefully to suspend all particles''; ''Failure to follow directions for use may lead to infection''.

Globin zinc insulin:

Caution—Do not remove stopper. Not for intravenous nor intramuscular use. Do not use after expiration date shown on outside wrapper or container. Do not use if any turbidity or precipitate has developed in the solution. Do not substitute for any other insulin-containing drug unless directed by physician.

In addition to the above warnings for globin zinc insulin, the following statements should be included in the labeling: ''Keep in a cold place, avoid freezing. Failure to follow directions for use may lead to infection''.

IPECAC SYRUP IN ONE-FLUID OUNCE CONTAINERS FOR EMERGENCY TREATMENT OF POISONING, TO INDUCE VOMITING. (See § 201.308 of this chapter.)

Ipecac syrup packaged for over-the-counter sale must bear statements to the following effect, in a prominent and conspicuous manner:

The following statement (boxed and in red letters):

''For emergency use to cause vomiting in poisoning. Before using, call

physician, the Poison Control Center, or hospital emergency room immediately for advice.''

The following warning: Warning—Keep out of reach of children. Do not use in unconscious persons. Ordinarily, this drug should not be used if strychnine, corrosives such as alkalies (lye) and strong acids, or petroleum distillates such as kerosene, gasoline, coal oil, fuel oil, paint thinner, or cleaning fluid have been ingested.

ISOAMYLHYRDOCUPREINE AND ZOLAMINE HYDROCHLORIDE RECTAL PREPARATIONS FOR EXTERNAL USE (See § 310.201(a)(3) of this chapter.)

Warning—Do not use this preparation in case of rectal bleeding, as this may indicate serious disease.

NEOMYCIN SULFATE WITH A VASOCONSTRICTOR, IN NASAL PREPARATIONS (SPRAY OR DROPS).

Caution—Do not exceed recommended dosage. Do not administer to children under 3 years of age unless directed by physician.

PRAMOXINE HYDROCHLORIDE FOR EXTERNAL USE. (See § 310.201(a)(19) of this chapter.)

Caution—Do not use in the eyes or nose. Not for prolonged use. Do not apply to large areas of the body. If redness, irritation, swelling, or pain persists or increases, discontinue use unless directed by a physician.

SODIUM GENTISATE. (See §§ 201.314, 310.201(a)(2) of this chapter.)

Warning—Do not give to children under 6 years of age or use for prolonged period unless directed by physician.

Warning—Keep this and all medications out of the reach of children; or

Warning—Keep out of the reach of children.

If offered for use in arthritis or rheumatism, in juxtaposition therewith, the statement:

Caution—If pain persists for more than 10 days, or redness is present, or in conditions affecting children under 12 years of age, consult a physician immediately.

TUAMINOHEPTANE SULFATE NASAL PREPARATIONS. (See § 310.201(a)(16) of this chapter.)

Caution—Do not exceed recommended dosage. Overdosage may cause nervousness, restlessness, or sleeplessness. Individuals with high blood pressure, heart disease, diabetes, or thyroid disease should use only as directed by physician. Do not use for more than 3 or 4 consecutive days unless directed by physician.

VIBESATE PREPARATIONS. (See § 310.201(a)(18) of this chapter.)

Caution—Do not use but consult physician for deep or puncture wounds or serious burns. If redness, irritation, swelling, or pain persists or increases, discontinue use and consult physician.

Warning—Contents under pressure. Do not puncture. Do not use or store near heat or open flame. Exposure to temperatures above 130° Fahrenheit may cause bursting. Never throw container into fire or incinerator.

[39 FR 11745, Mar. 29, 1974, as amended at 40 FR 8917, Mar. 3, 1975; 40 FR 13496, Mar. 27, 1975; 41 FR 10885, Mar. 15, 1976; 42 FR 22033, Apr. 29, 1977; 42 FR 36994, July 19, 1977; 44 FR 22053, Apr. 13, 1979; 44 FR 55170, Sept. 25, 1979; 52 FR 15893, Apr. 30, 1987; 52 FR 30057, Aug. 12, 1987; 52 FR 47324, Dec. 11, 1987; 55 FR 11582, Mar. 29, 1990; 57 FR 58376, Dec. 9, 1992; 59 FR 4218, Jan. 28, 1994; 61 FR 20101, May 3, 1996]

§ 369.22 Drugs; warning and caution statements specifically required by law.

PREPARATIONS CONTAINING HABIT-FORMING DERIVATIVES OF SUBSTANCES NAMED IN SECTION 502(d) OF THE ACT. (See §§ 329.1, 329.10, and 329.20 of this chapter.)

The statement ''*Warning*—May be habit forming'' is required to appear on the labels of all drugs containing derivatives designated in § 329.1 of this chapter as habit forming, including exempt narcotic preparations described in § 329.20(a) of this chapter and preparations containing one or more derivatives of barbituric acid, unless such drug is not suitable for internal use and is distributed and sold exclusively for such external use as involves no possibility of habit formation.

PART 429—DRUGS COMPOSED WHOLLY OR PARTLY OF INSULIN

Subpart A—General Provisions

Sec.
429.3 Definitions and interpretations.

Subpart B—Packaging and Labeling

429.10 Packaging.
429.11 Labeling.
429.12 Distinguishing colors on packages.

Subpart C—Product Standards

429.25 Standards of quality and purity for protamine.
429.26 Standards of quality and purity for globin hydrochloride.

Subpart D—Tests and Methods

429.30 Tests and methods of assay.

Subpart E—Certification

429.40 Requests for certification; samples; storage; approvals preliminary to certification.
429.41 Certifications.
429.45 Conditions on the effectiveness of certificates.
429.47 Authority to refuse certification service.

Subpart F—Administrative Procedures

429.50 Hearing procedure.
429.55 Fees.

Subpart G—Records

429.60 Records of distribution.

AUTHORITY: 21 U.S.C. 352, 356, 371.

CROSS REFERENCES: For other regulations in this chapter concerning insulin drugs, see also §200.15.

SOURCE: 39 FR 11750, Mar. 29, 1974, as amended at 40 FR 13497, Mar. 27, 1975, unless otherwise noted.

Subpart A—General Provisions

§429.3 Definitions and interpretations.

For the purpose of the regulations in this part:

(a) The term *act* means the Federal Food, Drug, and Cosmetic Act, as amended.

(b) The term *Secretary* means the Secretary of Health and Human Services.

(c) The term *Commissioner* means the Commissioner of Food and Drugs.

(d) The term *U.S.P.* means the official United States Pharmacopeia, including supplements thereto.

(e) The term *N.F.* means the official National Formulary, including supplements thereto.

(f) The definitions and interpretations of terms contained in section 201 of the act shall be applicable to such terms when used in the regulations in this part.

(g) The term *insulin* means the active principle of pancreas which affects the metabolism of carbohydrate in the animal body and which is of value in the treatment of diabetes mellitus.

(h) The term *insulin injection* means the insulin injection recognized in the U.S.P.

(i) The term *protamine zinc insulin suspension* means the protamine zinc insulin suspension recognized in the U.S.P.

(j) The term *globin zinc insulin injection* means the globin zinc insulin injection recognized in the U.S.P.

(k) The term *isophane insulin suspension* means the isophane insulin suspension recognized in the U.S.P.

(l) The term *insulin zinc suspension* means the insulin zinc suspension recognized in the U.S.P.

(m) The term *prompt insulin zinc suspension* means the prompt insulin zinc suspension recognized in the U.S.P.

(n) The term *extended insulin zinc suspension* means the extended insulin zinc suspension recognized in the U.S.P.

(o) The term *master lot* means a quantity (which is purified and which has been mixed in one container so as to be homogeneous) of:

(1) A concentrated solution of insulin; or

(2) The insulin-containing solids, in amorphous or crystalline form, derived from one or more such solutions.

(p) Except as provided in §429.41(c), the term *batch* means a quantity of a drug, in labeled packages, of uniform composition and intended for administration without further change, in which the sole insulin-containing ingredient is a single dilution (which has been mixed in one container so as to be homogeneous) of:

(1) A single master lot or part thereof; or

(2) A mixture of two or more master lots or parts thereof; except that such term means a portion of such quantity when certification of such portion is requested.

(q) The term *master lot mark* means an identifying mark or other identifying device assigned to a master lot by the manufacturer thereof.

(r) The term *batch mark* means an identifying mark or other identifying device assigned to a batch by the manufacturer thereof.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40285, Nov. 15, 1974]

Subpart B—Packaging and Labeling

§ 429.10 Packaging.

Each batch shall be packaged in immediate containers of colorless transparent glass. Such containers shall be closed with a substance through which successive doses may be withdrawn by hypodermic needle without removing the closure or destroying its effectiveness. The containers and closures shall be sterile at the time the containers are filled and closed. The composition of the containers and closures shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor prescribed in applicable standards of strength, quality, and purity. The shape of the containers shall be cylindrical, except that the cross-section of the containers for isophane insulin suspension containing less than 100 U.S.P. Units of insulin per milliliter shall be a rounded square, and the shoulder of the containers for insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension containing less than 100 U.S.P. Units of insulin per milliliter shall be hexagonal.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40285, Nov. 15, 1974]

§ 429.11 Labeling.

Each package from a batch that has been certified in accordance with the regulations in this part shall bear, on its label or labeling as hereinafter indicated, the following:

(a) On the outside wrapper or container and the immediate container of the retail package:

(1) The batch mark of such batch;

(2) The potency of the drug in terms of the U.S.P. Units of insulin per milliliter; and

(3) The statement ''Expiration date _____,'' the blank being filled in with the date on which the certificate applicable to such batch expires with respect to such package, as provided in § 429.45(b)(1).

(b) On the outside container or wrapper of the retail package, the statement ''Keep in a cold place, avoid freezing.''

(c) If the batch contains 40 or 100 U.S.P. Units of insulin per milliliter, on the circular or other labeling of the retail package:

(1) A statement that the treatment of diabetes mellitus is an individual problem and that the use of the drug, the time of its administration, and the number of daily doses and the quantity of each, as well as diet and exercise, are problems which require direct and continuous medical supervision;

(2) A statement explaining that the volume of the dose depends on the number of units of insulin per milliliter stated on the label, and that the patient should understand the meaning of the volume markings on the syringe;

(3) A description of a practicable method for sterilizing the needle and syringe before use;

(4) A description of the technique of withdrawal from the vial and the use of an antiseptic on the stopper, and a caution against the removal of the stopper;

(5) A description of the technique for cleansing, and the use of an antiseptic on the site of injection;

(6) A statement that failure to comply with the techniques described in paragraphs (c) (3), (4), and (5) of this section may lead to infection of the patient;

(7) A statement that injection should be subcutaneous, at a different site from that of the preceding injection, and a caution against intravenous or intramuscular use;

(8) An explanation of hypoglycemia and its relation to overdosage, omission of meals, illness, and infection;

(9) A statement of the significance of sugar in the urine and of the necessity of tests therefor; and

(10) A caution against use after the expiration date shown on the outside wrapper or container.

(d) On the circular or other labeling of the retail package, if the batch is insulin injection (in addition to the information required by paragraphs (a), (b), and (c) or (i) of this section), a caution against use if the drug has become viscous or if its color has become other than water clear.

(e) On the outside wrapper or container and immediate container of the retail package, if the batch is protamine zinc insulin suspension, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension (in addition to the information required by paragraphs (a), (b), and (c) of this section), the statement ''Shake carefully,'' or ''Shake well before using,'' or ''Shake well,'' or ''Shake carefully to suspend all particles.''

(f) On the circular or other labeling of the retail package, if the batch is protamine zinc insulin suspension, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension (in addition to the information required by paragraphs (a), (b), (c), and (e) of this section):

(1) An explanation of the difference, as compared with other insulin-containing drugs, in onset of action, duration, and the time and frequency of administration;

(2) A caution that it is not to be substituted for any other insulin-containing drug except on the advice and direction of a physician;

(3) A statement that a uniform suspension of the preparation is necessary and is brought about by careful shaking before use; and

(4) A caution against use when the precipitate has become lumped or granular in appearance or has formed a deposit of solid particles on the wall of the container.

(g) On the circular or other labeling of the retail package, if the batch is globin zinc insulin injection (in addition to the information required by paragraphs (a), (b), and (c) of this section):

(1) An explanation of the difference, as compared with other insulin-containing drugs, in onset of action, duration, and the time and frequency of administration;

(2) A caution that it is not to be substituted for any other insulin-containing drug, except on the advice and direction of a physician; and

(3) A caution against use if any turbidity or precipitate has developed in the solution.

(h) If the batch contains 500 U.S.P. Units of insulin per milliliter, on the outside container or wrapper and the immediate container of the retail package:

(1) The statement ''Caution: Federal law prohibits dispensing without prescription''; [2] and

(2) The statement ''Warning—High potency—Not for ordinary use''.

(i) If the batch contains 500 U.S.P. Units of insulin per milliliter, on the circular or other labeling of the retail package:

(1) Information adequate for the safe and effective use of the drug, by practitioners licensed by law to administer it, in insulin shock therapy and for the treatment of diabetic patients with high insulin resistance (daily requirement more than 200 units);

(2) A prominently placed and conspicuous statement: ''Warning—This insulin preparation contains 500 units of insulin in each milliliter. Extreme caution must be observed in measurement of dosage because inadvertent overdose may result in irreversible insulin shock. Serious consequences may result if it is used other than under constant medical supervision'';

(3) A caution against intravenous use; and

(4) A caution against use after the expiration date shown on the outside wrapper or container.

[39 FR 11750, Mar. 29, 1974, as amended at 40 FR 13497, Mar. 27, 1975; 41 FR 6912, Feb. 13, 1976; 44 FR 55170, Sept. 25, 1979]

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[2] For the Spanish-language version of the required labeling statement, see § 201.16(a), § 801.16 and § 290.6 of this chapter.

§ 429.12 Distinguishing colors on packages.

(a) The outside containers or wrappers of the packages, and the labels on the immediate containers of each potency of insulin injection shall be distinguished by the following colors:

Red, if it contains 40 U.S.P. Units of insulin per milliliter.
White, if it contains 100 U.S.P. Units of insulin per milliliter.
Narrow (at least 5 but not more than 20 to each inch) brown and white diagonal stripes, if it contains 500 U.S.P. Units of insulin per milliliter.

But if the master lot used was in crystalline form, the distinguishing colors, instead of those prescribed above, may be the following:

Red and gray, if it contains 40 U.S.P. Units of insulin per milliliter.

(b) The outside containers or wrappers of the packages, and the labels on the immediate containers of each potency of protamine zinc insulin suspension shall be distinguished by the following colors:

Red and white, if it contains 40 U.S.P. Units of insulin per milliliter.
Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(c) The outside containers or wrappers of the packages, and the labels of the immediate containers of each potency of globin zinc insulin injection shall be distinguished by the following colors:

Red and brown, if it contains 40 U.S.P. Units of insulin per milliliter.
Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(d) The outside containers or wrappers of the packages, and the labels of the immediate containers of each potency of isophane insulin suspension shall be distinguished by the following colors:

Red and blue, if it contains 40 U.S.P. Units of insulin per milliliter.
Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(e) The outside containers or wrappers of the packages, and the labels of the immediate containers, of insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension shall bear a mark or design to distinguish each drug, and each potency of these drugs shall be distinguished by the following colors:

Red and lavender, if it contains 40 U.S.P. Units of insulin per milliliter.
Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974; 44 FR 55170, Sept. 25, 1979]

Subpart C—Product Standards

§ 429.25 Standards of quality and purity for protamine.

When protamine is dried to constant weight at 100° C., its total nitrogen content is not less than 22.5 percent and not more than 25.5 percent, and its sulfate content, calculated as SO_4, is not less than 16 percent and not more than 19 percent.

§ 429.26 Standards of quality and purity for globin hydrochloride.

The ash content of globin hydrochloride is not more than 0.3 percent; its nitrogen content, calculated to moisture, ash, and hydrochloric acid free basis, is not less than 16.0 percent and not more than 17.5 percent.

Subpart D—Tests and Methods

§ 429.30 Tests and methods of assay.

The following tests and methods of assay are prescribed for the purposes of the regulations in this part 429. (All reagents specified in this section shall be of U.S.P. quality or better.)

(a) *Tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension.* The tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension shall be those set forth therefor in the U.S.P. or N.F., except that alternative test procedures may be employed when such have been authorized by the Commissioner.

(b) [Reserved]

(c) *Isophane ratio.* The isophane ratio shall be expressed as milligrams of protamine per 100 U.S.P. Units of insulin.

(1) *Reagents*—(i) *The stock buffer solution.* Dissolve in water the quantities of metacresol, phenol, glycerin, and disodium phosphate required to make 10 liters of the batch of isophane insulin and dilute to 1,000 milliliters.

(ii) *The insulin solution.* From a sample of the zinc-insulin crystals to be used in making the batch weigh a quantity which contains 10,000 U.S.P. Units of insulin. Dissolve the crystals in 15 milliliters of 0.1 percent hydrochloric acid. The resulting solution must be clear. Add it to 25 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). Dilute with water to approximately 200 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. The solution must be clear at this stage. If sodium chloride is to be used in preparing the batch add 25 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute to 250 milliliters with water. The pH must be between 7.1 and 7.4.

(iii) *The protamine solution.* Weigh 500 milligrams of the protamine to be used in making the batch and dissolve in 10 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). If sodium chloride is to be used in preparing the batch add 10 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute with water to approximately 80 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. Dilute with water to 100 milliliters. The pH must be between 7.2 and 7.4 and the solution must be clear.

(2) *Conduct of the test.* Measure six 25-milliliter samples of the insulin solution (paragraph (c)(1)(ii) of this section) into six tubes. To the first tube add 0.60 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section), to the second add 0.72 milliliter, to the third add 0.84 milliliter, to the fourth add 0.96 milliliter, to the fifth add 1.08 milliliters, and to the sixth add 1.20 milliliters. Mix the contents of each tube and let stand for at least 30 minutes. Centrifuge. (Do not filter.) From each supernatant fluid remove two 10-milliliter samples, thus creating two series of samples. To each of one series add 1 milliliter of the insulin solution (paragraph (c)(1)(ii) of this section). To each of the other series add 1 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section). Mix each sample and let stand 10 minutes. Measure the turbidity of each sample by means of a photometer or nephelometer. Plot the readings of the two series of samples, using the amount of protamine originally added in milligrams per 100 U.S.P. Units of insulin as abscissas, and the photometer or nephelometer readings as ordinates. The abscissa of the intersection of the two curves indicates the isophane ratio of the protamine to the zinc-insulin crystals. In order to increase the precision of the test, when the approximate isophane ratio is known, the quantities of protamine solution to be added to the six tubes may be so chosen that the range (0.60 to 1.20 milliliters) is reduced, and the approximate isophane ratio is near the middle of the range.

The isophane ratio found is not more than 100 percent nor less than 90 percent of the ratio of protamine to insulin used in the trial mixture referred to in § 429.40(d)(7).

(d)–(e) [Reserved]

(f) *Chloride in globin hydrochloride*—(1) *Conduct of the test.* Weigh accurately approximately 0.5 gram of globin hydrochloride into a small beaker and dissolve in 10–15 milliliters of distilled water. Add 10 milliliters of tenth-normal silver nitrate, 5 milliliters of nitric acid, and 5 milliliters of a saturated solution of potassium permanganate. Stir and place on a steam bath for approximately 1 hour. If any brown color remains, stir again, rinse the sides of the beaker with distilled water and place on the steam bath until the brown color disappears. Transfer quantitatively to a 50-milliliter volumetric flask and fill the flask to the mark with distilled water. Mix and filter through a dry filter paper into a dry vessel. Transfer exactly 40 milliliters of the filtrate to a flask, add 2 milliliters of ferric ammonium sulfate test solution and titrate with tenth-normal ammonium thiocyanate. To obtain the percent chloride as HCl, subtract 1.25 times the number of milliliters of ammonium thiocyanate used from 10;

multiply this difference by 0.365 and divide by the weight of the sample in grams.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(g) *Sulfate in protamine*—(1) *Conduct of the test.* Weigh accurately about 250 milligrams of protamine and dissolve it in about 100 milliliters of approximately tenth-normal hydrochloric acid. Heat to boiling and add 5 milliliters of barium chloride test solution. Digest on a steam bath for 1 hour; allow to cool. Filter through an ignited and weighed Gooch crucible; wash free of chlorides. Dry, ignite, and weight. The weight of barium sulfate thus obtained multiplied by 41.15 and divided by the weight of sample is the percent sulfate (SO_4) in the sample. Calculate the results to a moisture-free basis.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(h) *Nitrogen.* Determine total nitrogen by the method described in the U.S.P., for insulin U.S.P.

(i) *Zinc in insulin-containing solutions or suspensions.* Use the method described in the U.S.P. for insulin injection.

(j) *Zinc in insulin-containing solids.* Dissolve 10 to 20 milligrams, accurately weighed, of insulin-containing solids in 5 to 10 milliliters of distilled water containing one drop of 5*N* hydrochloric acid, and proceed as directed in the U.S.P. under the test for zinc in insulin injection.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974]

Subpart E—Certification

§ 429.40 Requests for certification; samples; storage; approvals preliminary to certification.

(a) A request for certification of a batch is to be addressed to the Food and Drug Administration, Division of Research and Testing (HFD–470), 200 C St. SW., Washington, DC 20204.

(b) The initial request for certification submitted by any person shall be preceded or accompanied by a full statement of the facilities and controls used to maintain the identity, strength, quality, and purity of each batch, including a description of:

(1) The equipment, methods, and processes used in diluting master lots and parts thereof, and in maintaining the identity, strength, quality, and purity of master lots and dilutions therefrom;

(2) The tests and assays made on master lots and mixtures thereof, on dilutions and batches therefrom, and on ingredients used in such dilutions and batches; and

(3) The laboratory facilities used in such controls.

Such initial request shall also be preceded or accompanied by the keys to the master lot marks and batch marks used by such person. When any change is made in any of such facilities or controls, or in any such key, the next request for certification thereafter shall be accompanied by a full statement of such change.

(c) A person who requests certification of a batch shall submit in connection with his request statements showing:

(1) The master lot mark of each master lot used or to be used wholly or partly as an ingredient or component of an ingredient of the batch;

(2) The quantity of each such master lot so used;

(3) The original quantity of each such master lot (unless such information has been previously submitted);

(4) The quantity of the batch; and

(5) The batch mark.

(d) Except as otherwise provided in paragraphs (g) and (h) of this section, a person who requests certification of a batch shall submit in connection with his request and in the quantities hereinafter indicated, accurately representative samples of the following:

(1) The single master lot or the mixture of two or more master lots or parts thereof, to be used as ingredients of the batch; in a quantity containing approximately 10,000 U.S.P. Units of insulin, except that, if the batch is to be isophane insulin suspension, the quantity shall contain not less than 20,000 U.S.P. Units of insulin.

(2) If the batch is to be insulin injection, a trial dilution made from such master lot or mixture, glycerin, phenol or cresol, and hydrochloric acid, which dilution conforms to the standard of identity, strength, quality, and purity

for insulin injection, except that it may contain approximately 40, 80, or 100 units of insulin per milliliter in a quantity containing approximately 5,000 U.S.P. units of insulin.

(3) If the batch is to be protamine zinc insulin suspension, a trial mixture which is intended to be accurately representative of the mixture which will constitute the finished batch; in a quantity containing approximately 10,000 U.S.P. units of insulin.

(4) If the batch is to be protamine zinc insulin suspension or isophane insulin suspension, the lot of protamine used as an ingredient of the trial mixture referred to in paragraph (d)(3) or (7) of this section; in a quantity of approximately 2 grams.

(5) If the batch is to be globin zinc insulin injection, a trial mixture made from the master lot or mixture referred to in paragraph (d)(1) of this section, globin, zinc chloride, hydrochloric acid, glycerin, and phenol or cresol, which mixture is intended to be accurately representative of the mixture which will constitute the finished batch; in a quantity containing approximately 10,000 U.S.P. units of insulin.

(6) If the batch is to be globin zinc insulin injection, the lot of globin hydrochloride from which the globin is to be prepared for use as an ingredient of the trial mixture referred to in paragraph (d)(5) of this section; in a quantity of approximately 5 grams.

(7) If the batch is to be isophane insulin suspension, a trial mixture which is intended to be accurately representative of the finished batch; in a quantity of approximately 10,000 U.S.P. units of insulin.

(8) If the batch is to be insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, a trial mixture which is intended to be accurately representative of the finished batch; in a quantity of approximately 50 milliliters.

(9) The finished batch; for all tests except sterility, not less than 10 retail packages.

(10) The finished batch for sterility testing, 20 retail packages, collected at approximately equal intervals throughout each filling operation (as defined by the U.S.P.), except that if it is insulin injection containing 500 U.S.P. Units of insulin per milliliter, in lieu of the volume contained in the retail package each such container may contain an amount of drug that is less than that contained in the retail package but in no case less than 5 milliliters.

(e) Except as otherwise provided by paragraphs (g) and (h) of this section, a person who requests certification shall submit in connection with his request results of the tests and assays listed after each of the following materials, made by him on a sample of such material:

(1) The master lot or mixture, referred to in paragraph (d)(1) of this section: Ash, nitrogen, potency, pH, sterility, and zinc, if such master lot or mixture is a solution; ash, moisture, nitrogen, potency, and zinc, if such master lot or mixture is a solid.

(2) A trial dilution of such master lot or mixture, of the potency of the trial dilution referred to in paragraph (d)(2) of this section: Nitrogen, pH, and potency.

(3) If the batch is to be protamine zinc insulin suspension, the trial mixture referred to in paragraph (d)(3) of this section: Nitrogen, pH, zinc, and biological reaction (by the test prescribed in the U.S.P.).

(4) If the batch is to be protamine zinc insulin suspension or isophane insulin suspension, the protamine referred to in paragraph (d)(4) of this section: Moisture, nitrogen, and sulfate.

(5) If the batch is to be globin zinc insulin injection the trial mixture referred to in paragraph (d)(5) of this section: Nitrogen, pH, zinc, and biological reaction (by the test prescribed in the U.S.P.).

(6) If the batch is to be globin zinc insulin injection, the globin hydrochloride referred to in paragraph (d)(6) of this section: Moisture, nitrogen, chloride, and ash.

(7) If the batch is to be isophane insulin suspension, the trial mixture referred to in paragraph (d)(7) of this section: Nitrogen, pH, zinc, isophane ratio of the protamine to the master lot or mixture (by the test prescribed in § 429.30(c)), and biological activity of the supernatant liquid (by the test prescribed in the U.S.P.).

(8) If the batch is to be insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, the trial mixture referred to in paragraph (d)(8) of this section: Nitrogen, pH, zinc, zinc in the supernatant liquid and insulin not extracted by buffered acetone solution.

(9) The finished batch: Nitrogen, pH, sterility; and if the batch is protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, zinc.

(f) The results of tests and assays for the following shall be reported in the terms indicated:

(1) Ash (except globin hydrochloride)—milligrams per 1,000 U.S.P. Units of insulin.

(2) Ash in globin hydrochloride—percent by weight.

(3) Chloride—percent by weight as HCl.

(4) Insulin not extracted by buffered acetone solution—percent of total nitrogen of the preparation not extracted by buffered acetone solution.

(5) Isophane ratio—milligrams of protamine per 100 U.S.P. Units of insulin.

(6) Moisture—percent by weight.

(7) Nitrogen (except in globin hydrochloride and protamine)—milligrams per milliliter in the cases of solutions and suspensions, and percent by weight in the case of solids.

(8) Nitrogen in globin hydrochloride—percent by weight, calculated to a moisture-free, ash-free, chloride-free basis.

(9) Nitrogen in protamine—percent by weight, calculated to a moisture-free basis.

(10) Potency—U.S.P. Units of insulin per milliliter in the case of solutions, and U.S.P. Units of insulin per milligram in the case of solids.

(11) pH.

(12) Sulfate—percent by weight as SO_4, calculated to a moisture-free basis.

(13) Zinc—milligrams per milliliter in the cases of solutions and suspensions, and percent by weight in the case of solids.

(g)(1) No sample referred to in paragraphs (d) (1) to (3), inclusive, of this section, and no result referred to in paragraphs (c) (1) to (8), inclusive of this section, is required if such sample or result has been submitted in connection with a previous request for certification. Except for paragraphs (d) (9), (10), and (e)(9), the samples referred to in paragraph (d) of this section and the results referred to in paragraph (e) of this section for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, or isophane insulin suspension are not required if the Commissioner has previously approved a trial mixture containing 40, 100 units of insulin per milliliter or trial dilution containing approximately 40, 100 units of insulin per milliliter and the mixture or dilution was prepared from the same materials and in the same manner, except for adjustment of pH of the buffer solution.

(2) Each sample submitted pursuant to this section shall be so packaged as to maintain its representative character, and in the case of any solution or suspension, shall be collected and packaged under aseptic conditions. Each package shall be clearly identified as to its contents and shall bear the name and post office address of the person submitting the request.

(3) The packages constituting the samples submitted pursuant to paragraph (d)(9) of this section shall be collected at such intervals that the quantities packaged between collections are approximately equal; in no case shall any such quantity be more than 10,000 packages. The collections shall cover the entire period of packaging.

(4) Each sample submitted pursuant to paragraphs (d) (2), (3), (5), (7), and (8) of this section shall be accompanied by a statement showing the identity, quality, and quantity of each substance used as an ingredient or as a component of an ingredient in the material from which the sample was taken.

(5) If the tests and assays, results of which are submitted pursuant to paragraph (e)(2) of this section, were not made on the same trial dilution as that from which the sample submitted pursuant to paragraph (d)(2) of this section was taken, such sample shall be accompanied by a statement showing the identity, quality, and quantity of each substance used as an ingredient or as a

component of an ingredient of the trial dilution on which such tests and assays were made.

(6) The value for nitrogen submitted pursuant to paragraphs (e) (1) and (2) of this section may be calculated from the result of a test therefor submitted pursuant to either paragraph (e) (1) or (2) of this section. The result on potency required under paragraph (e)(1) of this section may be calculated from an assay therefor submitted pursuant to paragraph (e)(2) of this section. The value of each of the components nitrogen and zinc, to the extent required under paragraph (e)(9) of this section, may be calculated from the result of a test therefor submitted pursuant to paragraph (e) (3), or (5), or (7) or (8) of this section or from the result of a test of the bulk dilution from which the batch was prepared. The value for nitrogen required under paragraph (e)(9) of this section may, if the batch is insulin injection, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension, be calculated from a test therefor submitted pursuant to either paragraph (e) (1) or (2) of this section. Each calculated value shall be indicated as such.

(7) The information required under paragraphs (c) (1), (2), and (3) of this section, and the samples and results of tests and assays required under paragraphs (d) (1) and (2) and (e) (1) and (2) of this section, should be submitted before submission of the samples and results required in paragraphs (d) (3) to (8), inclusive, of this section and (e) (3) to (8), inclusive, of this section; and the samples and results required under paragraphs (d) (3) to (8), inclusive, and (e) (3) to (8), inclusive, should be submitted before submission of the information, samples, and results required under paragraphs (c) (4) and (5), (d) (9) and (10), and (e)(9) of this section. All information, including results of tests and assays (except results of tests for sterility), required under this section should be submitted at the same time as the samples to which they relate are submitted.

(h) The person who requests certifications shall submit such information additional to that submitted pursuant to paragraphs (b), (c), (e), and (g) of this section, such additional samples of any substance referred to in paragraph (d) of this section, and such samples of any other substance used or to be used as an ingredient or as a component of an ingredient in the batch, as the Commissioner may require for the purpose of investigations to determine whether or not such batch complies with the requirements set forth by § 429.41 for the issuance of a certificate.

(i) After a sample required by paragraph (d) of this section is taken from any master lot or mixture of part of two or more master lots, such master lot or master lots and all parts thereof, and all dilutions and batches and all parts thereof in which any such master lot is used as an ingredient or as a component of an ingredient, shall be stored at the establishment where manufactured until used up or shipped or otherwise delivered, at a temperature above freezing but not above 15° C. (59° F.), and under such other conditions as prevent, so far as practicable, any change in composition; except that master lots and parts thereof which are solids may be stored at ordinary room temperatures.

(j) As promptly as practicable after the samples submitted pursuant to paragraphs (d) (1) and (2) of this section, and any other material or information relative thereto that may be required under this section, are received by the Commissioner, he shall notify the person who submitted such samples of his approval or refusal to approve the use of the master lot or mixture for the making of bulk dilutions. In case of a refusal to approve, the Commissioner shall state his reasons therefor.

(k) In like manner, the Commissioner shall notify the person who submits samples pursuant to paragraphs (d) (3) to (8), inclusive, of this section of his approval or refusal to approve the use of the materials represented by such samples in completing the manufacture of the batch. In case of a refusal to approve, the Commissioner shall state his reasons therefor.

(l) If, under the provisions of paragraph (j) or (k) of this section, the Commissioner has refused to approve any material for use in a subsequent operation, he shall examine no other

sample required hereunder which includes such material as an ingredient or component of an ingredient, unless and until the person requesting certification makes an adequate showing that the cause for such refusal no longer exists.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974; 44 FR 48968, Aug. 21, 1979; 44 FR 55170, Sept. 25, 1979; 45 FR 40111, June 13, 1980; 50 FR 8996, Mar. 6, 1985; 55 FR 11582, Mar. 29, 1990]

§ 429.41 Certifications.

(a) If it appears to the Commissioner, after such investigation as he considers necessary, that:

(1) The information (including results of tests and assays) and the samples required by or pursuant to § 429.40 have been submitted, and such information contains no untrue statement of a material fact;

(2) The batch complies with the regulations in this part 429 and conforms to the standards of identity, quality, strength, and purity for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension;

the Commissioner shall certify that such batch is safe and efficacious for use, subject to such conditions on the effectiveness of such certifications as are set forth in § 429.45, and shall issue to the person who requested it a certificate to that effect.

(b) If the Commissioner determines, after such investigation as he considers to be necessary, that the information submitted pursuant to § 429.40 or the batch covered by such request, does not comply with the requirements set forth in paragraph (a) of this section for the issuance of a certificate, the Commissioner shall refuse to certify such batch and shall give notice thereof to the person who requested certification, stating his reasons for refusal.

(c) Upon the request of the manufacturer, the Commissioner shall certify as a ''batch'' a master lot, which has been approved in accordance with § 429.40(j) as safe and efficacious for use in preparation of an insulin-containing drug, subject to the conditions on the effectiveness of such certifications as are set forth in § 429.45(a) (1) and (b) (4).

(d) For the purposes of his investigations under the authority of this section, the Commissioner may accept, when he is satisfied as to the completeness and accuracy thereof, the results of any tests or assays made by the control laboratory of the Insulin Committee of the University of Toronto.

§ 429.45 Conditions on the effectiveness of certificates.

(a) A certificate shall not become effective:

(1) If it is obtained through fraud, or through misrepresentation or concealment of a material fact.

(2) With respect to any package, unless its immediate container complies with the requirements of § 429.10 and such package or such immediate container has been so sealed that its contents cannot be used without destroying such package or seal.

(3) With respect to any package, unless its label and labeling bear all words, statements, and other information, and are distinguished by the color or colors, required by §§ 429.11 and 429.12.

(b) A certificate shall cease to be effective: (1) With respect to any package of insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, or extended insulin zinc suspension on the expiration date specified in the U.S.P.

(2) With respect to any package, when such package or the seal thereof or the immediate container therein or the seal of the immediate container is broken, or when its label or labeling ceases to conform to any requirement of § 429.11 or § 429.12.

(3) With respect to any package, when the drug therein so changes that it fails to meet the standards of identity, strength, quality, and purity upon the basis of which the batch was certified; except that those minor changes in potency (not exceeding 10 percent from the potency stated on the label, in the case of insulin injection) which occur before the expiration date, and which are normal and unavoidable in

good storage and distribution practice, shall be disregarded.

(4) With respect to a master lot of insulin, 5 years after date of issue if the master lot is a solution, or 10 years after date of issue if the master lot is a solid.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974]

§429.47 Authority to refuse certification service.

When the Commissioner finds, after giving notice and opportunity for hearing, that a person has:

(a) Obtained or attempted to obtain a certificate through fraud, or through misrepresentation or concealment of a material fact;

(b) Falsified the records required to be kept by §429.60; or

(c) Failed to keep such records or to make them available, or to accord full opportunity to make an inventory of stocks on hand or otherwise to check the correctness of such records, as required by such section;

the Commissioner may immediately suspend service to such person under the regulations in this part, and may continue such suspension unless and until such person shows adequate cause why such suspension should be terminated.

Subpart F—Administrative Procedures

§429.50 Hearing procedure.

Hearings pursuant to §429.47 shall be governed by part 16 of this chapter.

[41 FR 48267, Nov. 2, 1976, as amended at 42 FR 15674, Mar. 22, 1977]

§429.55 Fees.

(a)(1) Fees for the services rendered under the regulations in this part shall be such as are necessary to provide, equip, and maintain an adequate certification service.

(2) Whenever in the judgment of the Commissioner the ratio between fees collected (which are based upon experience and the best estimate of costs and the best estimate of earnings) and the costs of providing the service during an elapsed period of time, in the light of all circumstances and contingencies, warrants a refund from the fund collected during such period, he shall make ratable refunds to those persons to whom the services were rendered and charged.

(b) The fees for requests for certification submitted under §429.40 are as follows:

(1) $2,400 for each master lot or mixture of two or more master lots or parts thereof.

(2) $1,700 for each dosage form batch.

(3) The fees established in this paragraph may increase as Federal salary costs increase. The rate of increase will be no higher than Federal salary increases, commencing with pay raises on or after January 1, 1997. Notification of the exact fees established and adjustments will be communicated directly to the manufacturers of insulin products.

(c) A person requiring continuing certification services may maintain an advance deposit of the estimated costs of such services for a period of 2 months or more. Such deposits shall be debited with fees for services rendered, but shall not be debited for any fee the amount of which is not definitely specified in these regulations unless the depositor has previously requested the performance of the services to be covered by such fee. A monthly statement for each such advance deposit shall be rendered.

(d) The unearned portion of any advance deposit made pursuant to paragraph (b) or (c) of this section shall be refunded to the depositor upon his application.

(e) All advance deposits required by the regulations in this part 429 shall be paid by money order, bank draft, or certified check drawn to the order of the Food and Drug Administration, collectible at par at Washington, DC. All deposits shall be forwarded to the Food and Drug Administration, Department of Health and Human Services, Washington, DC 20204, whereupon after making appropriate record thereof they will be transmitted to the Chief Disbursing Officer, Division of Disbursement, Treasurer of the United

States, for deposit to the special account ''Salaries and Expenses, Certification, Inspection and Other Services, Food and Drug Administration.''

[39 FR 11750, Mar. 29, 1974, as amended at 42 FR 27227, May 27, 1977; 48 FR 788, Jan. 7, 1983; 60 FR 56516, Nov. 9, 1995]

Subpart G—Records

§ 429.60 Records of distribution.

(a) The person to whom a certificate is issued shall keep complete records showing each shipment and other delivery (including exports) of each batch or part thereof, by the person requesting certification, and showing each such shipment and delivery into, or from any place in, any State or Territory, made by any person subject to his control, including records showing the date and quantity of each such shipment and delivery and the name and post office address of the person to whom such shipment or delivery was made.

(b) Upon the request of any officer or employee of the Food and Drug Administration or of any other officer or employee of the United States, acting on behalf of the Secretary, the person to whom a certificate is issued, at all reasonable hours within 2 years after disposal of all the batch covered by such certificate, shall make such records available to any such officer or employee, and shall accord to such officer or employee full opportunity to make inventory of stocks of such batch on hand and otherwise to check the correctness of such records.

PART 430—ANTIBIOTIC DRUGS; GENERAL

Subpart A—General Provisions

Sec.
430.3 Definitions applicable to all certifiable antibiotic drugs.
430.4 Definitions of antibiotic substances.
430.5 Definitions of master and working standards.
430.6 Definitions of the terms ''unit'' and ''microgram'' as applied to antibiotic substances.

Subpart B—Antibiotic Drugs Affected by the Drug Amendments of 1962

430.10 Certification or release of antibiotic drugs affected by the drug amendments of 1962.

AUTHORITY: 21 U.S.C. 321, 351, 352, 353, 355, 357, 371; 42 U.S.C. 216, 241, 262.

Subpart A—General Provisions

§ 430.3 Definitions applicable to all certifiable antibiotic drugs.

(a) The definitions and interpretations contained in section 201 of the Federal Food, Drug, and Cosmetic Act shall be applicable to such terms when used in the regulations in this chapter covering the certification of antibiotic and antibiotic-containing drugs.

(b) The term *Commissioner* means the Commissioner of Food and Drugs and any other officer of the Food and Drug Administration whom he may designate to act in his behalf for the purpose of the regulations for the certification of antibiotic and antibiotic-containing drugs.

(c) The term *act* means the Federal Food, Drug, and Cosmetic Act and amendments thereto. (52 Stat. 1040 *et seq.*; 21 U.S.C. 301–392).

(d) The term *U.S.P.* means the official Pharmacopeia of the United States, including supplements thereto. The term *N.F.* means the official National Formulary, including supplements thereto.

(e) The term *batch* means a specific homogeneous quantity of a drug.

(f) The term *batch mark* means an identifying mark or other identifying device assigned to a batch by the manufacturer or packer thereof.

(g) The term *manufacture* does not include the use of a drug as an ingredient in compounding any prescription issued by a practitioner licensed by law to administer such drug.

[39 FR 18925, May 30, 1974]

§ 430.4 Definitions of antibiotic substances.

(a) The following are definitions of antibiotic substances:

(1) *Penicillin.* Each of the several antibiotic substances (e.g., penicillin F, penicillin G, penicillin X) produced by the growth of *Penicillium notatum* or *Penicillium chrysogenum,* and each of

the same substances produced by any other means, is a kind of penicillin.

(2) *Streptomycin.* Each of the several antibiotic substances produced by the growth of *Streptomyces griseus,* and each of the same substances produced by any other means, is a kind of streptomycin.

(3) *Dihydrostreptomycin.* Each of the antibiotic substances produced by hydrogenation of streptomycin, and each of the same substances produced by any other means, is a kind of dihydrostreptomycin.

(4) *Chlortetracycline.* Each of the several antibiotic substances produced by the growth of *Streptomyces aureofaciens,* and each of the same substances produced by any other means is a kind of chlortetracycline.

(5) *Tetracycline.* Each of the several antibiotic substances produced by the hydrogenation of chlortetracycline, and each of the same substances produced by any other means, is a kind of tetracycline.

(6) *Chloramphenicol.* Each of the several antibiotic substances produced by the growth of *Streptomyces venezuelae,* and each of the same substances produced by any other means, is a kind of chloramphenicol.

(7) *Bacitracin.* Each of the several antibiotic substances produced by the growth of *Bacillus subtilis* var. Tracy, and each of the same substances produced by any other means, is a kind of bacitracin.

(8) [Reserved]

(9) *Amphotericin.* Each of the antibiotic substances produced by the growth of *Streptomyces nodosus,* and each of the same substances produced by any other means, is a kind of amphortericin.

(10) *Colistin.* Each of the antibiotic substances produced by the growth of *Bacillus polymyxa* var. *colistinus,* and each of the same substances produced by any other means, is a kind of colistin.

(11) *Cycloserine.* Each of the antibiotic substances produced by the growth of *Streptomyces orchidaceus,* and each of the same substances produced by any other means, is a kind of cycloserine.

(12) *Erythromycin.* Each of the antibiotic substances produced by the growth of *Streptomyces erythreus,* and each of the same substances produced by any other means, is a kind of erythromycin.

(13) *Gramicidin.* Each of the antibiotic substances produced by the growth of *Bacillus brevis,* and each of the same substances produced by any other means, is a kind of gramicidin.

(14) *Griseofulvin.* Each of the antibiotic substances produced by the growth of *Penicillium patulum* or *Penicillium griseofulvum,* and each of the same substances produced by any other means, is a kind of griseofulvin.

(15) *Kanamycin.* Each of the antibiotic substances produced by the growth of *Streptomyces kanamyceticus,* and each of the same substances produced by any other means, is a kind of kanamycin.

(16) *Neomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces fradiae,* and each of the same substances produced by any other means, is a kind of neomycin.

(17) *Novobiocin.* Each of the antibiotic substances produced by the growth of *Streptomyces niveus* (known also as *Streptomyces spheroides*), and each of the same substances produced by any other means, is a kind of novobiocin.

(18) *Nystatin.* Each of the antibiotic substances produced by the growth of *Streptomyces noursei,* and each of the same substances produced by any other means, is a kind of nystatin.

(19) *Oleandomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces antibioticus,* and each of the same substances produced by any other means, is a kind of oleandomycin.

(20) *Troleandomycin.* Each of the antibiotic substances produced by the triacetylation of oleandomycin, and each of the same substances produced by any other means, is a kind of troleandomycin.

(21) *Oxytetracycline.* Each of the antibiotic substances produced by the growth of *Streptomyces rimosus,* and each of the same substances produced by any other means, is a kind of oxytetracycline.

(22) *Paromomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces rimosus* var. *paromomycinus,* and each of the same

substances produced by any other means, is a kind of paromomycin.

(23) *Polymyxin.* Each of the antibiotic substances produced by the growth of *Bacillus polymyxa,* and each of the same substances produced by any other means, is a kind of polymyxin.

(24) *Plicamycin.* Each of the antibiotic substances produced by the growth of a variant of *Streptomyces plicatus,* and each of the same substances produced by any other means, is a kind of plicamycin.

(25) *Tyrothricin.* Each of the mixtures of antibiotic substances produced by the growth of *Bacillus brevis,* and each of the same mixtures of substances produced by any other means, is a kind of tyrothricin.

(26) *Vancomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces orientalis,* and each of the same substances produced by any other means, is a kind of vancomycin.

(27) [Reserved]

(28) *Gentamicin.* Each of the antibiotic substances produced by the growth of *Micromonospora purpurea,* and each of the same substances produced by any other means, is a kind of gentamicin.

(29) *Dactinomycin.* Dactinomycin is a specific kind of actinomycin produced by the growth of *Streptomyces parvullus* or the same antibiotic produced by any other means.

(30) *Candicidin.* Each of the heptaene antibiotic substances produced by the growth of *Streptomyces griseus* and each of the same substances produced by any other means is a kind of candicidin.

(31) *Cephalosporin.* Each of the antibiotic substances produced by the growth of *Cephalosporium acremonium,* and each of the same substances produced by any other means, is a kind of cephalosporin.

(32) *Lincomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis,* and each of the same substances produced by any other means, is a kind of lincomycin.

(33) *Demeclocycline.* Each of the antibiotic substances produced by removal of the 6-methyl group from chlortetracycline, and each of the same substances produced by any other means, is a kind of demeclocycline.

(34) *Clindamycin.* Each of the antibiotic substances produced by the 7-chloro-substitution of the 7(R)hydroxyl group of lincomycin, and each of the same substances produced by any other means, is a kind of clindamycin.

(35) [Reserved]

(36) *Capreomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces capreolus,* and each of the same substances produced by any other means, is a kind of capreomycin.

(37) *Rifamycin.* Each of the several antibiotic substances (e.g., rifamycin A, rifamycin B, rifamycin SV) produced by the growth of *Streptomyces mediterranei,* and each of the same substances produced by any other means, is a kind of rifamycin.

(38) *Spectinomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces spectabilis,* and each of the same substances produced by any other means, is a kind of spectinomycin.

(39) *Mitomycin.* Mitomycin is the antibiotic substance produced by the growth of *Streptomyces caespitosus,* and each of the same substances produced by any other means is a kind of mitomycin.

(40) *Doxorubicin.* Each of the antibiotic substances produced by the growth of *Streptomyces peucetius* var. *caesius,* and each of the same substances produced by any other means, is a kind of doxorubicin.

(41) *Bleomycin.* Each of the antibiotic substances produced by the growth of *Streptomyces verticillus* and each of the same substances produced by any other means is a kind of bleomycin.

(42) *Tobramycin.* A specific one of the antibiotic substances produced by the growth of *Streptomyces tenebrarius,* and the same substance produced by any other means, is tobramycin.

(43) *Amikacin.* Each of the antibiotic substances produced by the acylation of the 1-amino group of the 2-deoxystreptamine moiety of kanamycin A with L-(-)-γ)amino-α-hydroxybuyric acid, and each of the same substances produced by any other means is a kind of amikacin.

(44) *Vidarabine.* Vidarabine is a purine glycoside antibiotic substance produced by the growth of *Streptomyces antibioticus,* and each of the same substances produced by any other means is a kind of vidarabine.

(45) *Natamycin.* Each of the antibiotic substances produced by the growth of *Streptomyces natalensis,* and each of the same substances produced by any other means, is a kind of natamycin.

(46) *Daunorubicin.* Each of the antibiotic substances produced by the growth of *Streptomyces coeruleorubidus* and each of the same substances produced by any other means is a kind of daunorubicin.

(47) *Sisomicin.* A specific one of the antibiotic substances produced by the growth of *Micromonospora inyoensis,* and the same substance produced by any other means, is a kind of sisomicin.

(48) *Moxalactam.* 5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[carboxy(4-hydroxyphenyl)acetyl]-amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]-methyl]-8-oxo-, disodium salt.

(49) *Cefoperazone.* Cefoperazone is a semi-synthetic antibiotic substance produced by the acylation of the amino group at the 7 position of 7-aminocephalosporanic acid with α-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-α-(4-hydroxyphenyl) acetic acid and introduction of a methylthiotetrazol group at the 3 position.

(50) *Netilmicin.* Netilmicin is a semi-synthetic antibiotic of the aminoglycoside group derived from sisomicin, and each of the same substances produced by any other means is a kind of netilmicin. It is D-Streptamine, 4-*O*-[3-amino-6-(aminomethyl)-3,4-dihydro-2*H*-pyran-2-yl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-N^1-ethyl-, (2*S*-*cis*)-.

(51) *Cyclosporine.* Cyclosporine is a specific cyclic polypeptide consisting of 11 amino acids produced by the growth of *Cylindrocarpon lucidum* Booth or *Tolypocladium inflatum* Gams.

(52) *Cefonicid.* 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(hydroxyphenylacetyl)amino]-8-oxo-3[[[1-(sulfomethyl)-1*H*-tetrazol-5yl]-thio]methyl]-, disodium salt, [6R-[6α7β(R*)]].

(53) *Clavulanic acid.* Clavulanic acid is an antibiotic substance produced by the growth of *Streptomyces clavuligerus* having the structure described as follows: *Z*-(2*R*, 5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, and each of the same substances produced by any other means, is a kind of clavulanic acid.

(54) *Ceftriaxone.* Ceftriaxone is a semi-synthetic antibiotic substance produced by the addition of S-2-benzothiazolyl-2-(2-aminothiazol-4-yl)-2-methoxyiminothioacetate to the 7 amino group of 7-amino-3-(2,5-dihydro-2 methyl-5,6-dioxo-1,2,4-triazin-3-yl)-thiomethyl-3-cephem-4-carboxylic acid.

(55) *Imipenem monohydrate.* Imipenem monohydrate is an antibiotic substance having the chemical structure described by the following name: [5*R*-[5α,6α,(R*)]]-6-(1-hydroxyethyl)-3-[[2-[(iminomethyl)amino]ethyl]thio]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic acid monohydrate.

(56) *Aztreonam.* [2*S*[2*alpha*,3*beta*(*Z*)]]-2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methylpropanoic acid.

(57) *Sulbactam.* Sulbactam is a semi-synthetic antibiotic substance produced by the oxidation of the sulfur atom at the 4 position to its dioxide and the deamination at the 6 position of (2*S*,5*R*)-6-amino-3,3-dimethyl-7-oxo-4 thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA).

(58) *Cefmenoxime.* Cefmenoxime is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, [6*R*-[6-α,7-β(Z)]]-.

(59) *Cefixime.* Cefixime is a semi-synthetic antibiotic substance produced by the acylation of the amino group at the 7 position of 7-aminocephalosporanic acid with a β-[(2-amino-4-thiazolyl)(carboxymethoxy)imino] acetyl group and the introduction of a vinyl group at the 3 position.

(60) *Cefotiam.* Cefotiam is an antibiotic substance having the chemical structure described by the following

name: 7-(*R*)-[2-(2-amino-4-thiazol)acetamido]-3-[[[1-(2-dimethylamino)ethyl]-1*H*-tetrazol-5-y1] thio] methyl]-3-cephem-4-carboxylic acid.

(61) *Mupirocin*. Each of the antibiotic substances produced by the growth of *Pseudomonas fluorescens*, and each of the same substances produced by any other means, is a kind of mupirocin.

(62) *Cefmetazole*. Cefmetazole is an antibiotic substance having the chemical structure described by the following name: (6*R*-cis)-7-[[[cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[(1-methyl-1*H*- tetrazol-5-yl)thio]methyl]-8-oxo-5- thia-1-azabicyclo[4.2.0]oct-2- ene-2-carboxylic acid.

(63) *Cefpiramide*. Cefpiramide is an antibiotic substance having the chemical structure described by the following name: (6*R*, 7*R*)-7-[(*R*)-2-(4-hydroxy-6-methyl-nicotinamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

(64) *Clarithromycin*. Clarithromycin is 6-O-methylerythromycin A.

(65) *Azithromycin*. Azithromycin is an antibiotic substance having the chemical structure described by the following name: (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-*L*-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

(66) *Cefprozil*. Cefprozil is an antibiotic substance having the chemical structure described by the following name: (6*R*,7*R*)-7-[(*R*)-2-amino-2-(*p*-hydroxyphenyl)acetamido]8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a mixture of the Z (cis) and E (trans) isomers in an approximate ratio of 9:1, respectively.

(67) *Idarubicin*. Idarubicin is an anthracycline antibiotic substance having the chemical structure described by the following name: 5,12-Naphthacenedione, 9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-*L*-*lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-(7*S*-*cis*).

(68) *Loracarbef*. Loracarbef is an antibiotic substance having the chemical structure described by the following name: (6*R*,7*S*)-7-[(*R*)-2-amino-2-phenylacetamido]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

(69) *Rifabutin*. Rifabutin is an antibiotic substance having the chemical structure described by the following name: (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,22*E*, 24*Z*)-6,16, 18,20-tetrahydroxy-1′-isobutyl-14-methoxy-7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8]naphth[1,2-d]imidazole-2,4′-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate.

(70) *Cefpodoxime proxetil*. Cefpodoxime proxetil is an antibiotic substance having the chemical structure described by the following name: (±)-1-Hydroxyethyl(+)-(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,7²-(*Z*)-(*O*-methyloxime), isopropyl carbonate (ester).

(b) [Reserved]

[39 FR 18925, May 30, 1974]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 430.4, see the List of CFR Sections Affected in the Finding Aids section of this volume.

§ 430.5 Definitions of master and working standards.

(a) *Master standards*—(1) *Penicillinand salts of penicillin*—(i) *Penicillin G* The term ''penicillin G master standard' means a specific lot of crystalline penicillin G that is designated by the Commissioner as the standard of comparison in determining the potency of the penicillin G working standard.

(ii) [Reserved]

(iii) *Penicillin V*. The term ''penicillin V master standard'' means a specific lot of crystalline penicillin V that is designated by the Commissioner as the standard of comparison in determining the potency of the penicillin V working standard.

(iv)–(v) [Reserved]

(vi) *Methicillin*. The term ''methicillin master standard'' means a specific lot of crystalline methicillin that is designated by the Commissioner

as the standard of comparison in determining the potency of the methicillin working standard.

(vii) *Oxacillin.* The term ''oxacillin master standard'' means a specific lot of crystalline oxacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the oxacillin working standard.

(viii) *Ampicillin.* The term ''ampicillin master standard'' means a specific lot of crystalline ampicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the ampicillin working standard.

(ix) *Nafcillin.* The term ''nafcillin master standard'' means a specific lot of crystalline nafcillin that is designated by the Commissioner as the standard of comparison in determining the potency of the nafcillin working standard.

(x) *Cloxacillin.* The term ''cloxacillin master standard'' means a specific lot of crystalline cloxacillin that is designated as the standard of comparison in determining the potency of the cloxacillin working standard.

(xi) *Dicloxacillin.* The term ''dicloxacillin master standard'' means a specific lot of dicloxacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the dicloxacillin working standard.

(xii) The term ''hetacillin working standard'' means a specific lot of homogenous preparation of hetacillin.

(2) *Streptomycin.* The term ''streptomycin master standard'' means a specific lot of streptomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the streptomycin working standard.

(3) *Dihydrostreptomycin.* The term ''dihydrostreptomycin master standard'' means a specific lot of crystalline dihydrostreptomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the dihydrostreptomycin working standard.

(4) *Chlortetracycline.* The term ''chlortetracyclin master standard'' means a specific lot of crystalline chlortetracycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the chlortetracycline working standard.

(5) *Demeclocycline.* The term ''demeclocycline master standard'' means a specific lot of crystalline demeclocycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the demeclocycline working standard.

(6) *Tetracycline.* The term ''tetracycline master standard'' means a specific lot of crystalline tetracycline hydrochloride that is designated by the Commissioner as the standard of comparison in determining the potency of the tetracycline working standard.

(7) *Rolitetracycline.* The term ''rolitetracycline master standard'' means a specific lot of crystalline rolitetracycline that is designated by the Commissioner as the standard of comparison in determining the potency of the rolitetracycline working standard.

(8) *Chloramphenicol.* The term ''chloramphenicol master standard'' means a specific lot of crystalline chloramphenicol that is designated by the Commissioner as the standard of comparison in determining the potency of the chloramphenicol working standard.

(9) *Bacitracin* The term ''bacitracin master standard'' means a specific lot of bacitracin that is designated by the Commissioner as the standard of comparison in determining the potency of the bacitracin working standard.

(10) [Reserved]

(11) *Amphotericin.* The term ''amphotericin A master standard'' means a specific lot of amphotericin A designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin A working standard. The term ''amphotericin B master standard'' means a specific lot of amphotericin B designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin B working standard.

(12) *Colistin.* The term ''colistin master standard'' means a specific lot of colistin designated by the Commissioner as the standard of comparison in

determining the potency of the colistin working standard.

(13) *Colistimethate.* The term ''colistimethate master standard'' means a specific lot of colistimethate designated by the Commissioner as the standard of comparison in determining the potency of the colistimethate working standard.

(14) *Cycloserine.* The term ''cycloserine master standard'' means a specific lot of cycloserine designated by the Commissioner as the standard of comparison in determining the potency of the cycloserine working standard.

(15) *Erythromycin.* The term ''erythromycin master standard'' means a specific lot of erythromycin designated by the Commissioner as the standard of comparison in determining the potency of the erythromycin working standard.

(16) *Gramicidin.* The term ''gramicidin master standard'' means a specific lot of gramicidin designated by the Commissioner as the standard of comparison in determining the potency of the gramicidin working standard.

(17) *Griseofulvin.* The term ''griseofulvin master standard'' means a specific lot of griseofulvin designated by the Commissioner as the standard of comparison in determining the potency of the griseofulvin working standard.

(18) *Kanamycin.* The term ''kanamycin master standard'' means a specific lot of kanamycin designated by the Commissioner as the standard of comparison in determining the potency of the kanamycin working standard.

(19) *Neomycin.* The term ''neomycin master standard'' means a specific lot of neomycin designated by the Commissioner as the standard of comparison in determining the potency of the neomycin working standard.

(20) *Novobiocin.* The term ''novobiocin master standard'' means a specific lot of novobiocin designated by the Commissioner as the standard of comparison in determining the potency of the novobiocin working standard.

(21) *Nystatin.* The term ''nystatin master standard'' means a specific lot nystatin designated by the Commissioner as the standard of comparison in determining the potency of the nystatin working standard.

(22) *Oleandomycin.* The term ''oleandomycin master standard'' means a specific lot of oleandomycin designated by the Commissioner as the standard of comparison in determining the potency of the oleandomycin working standard.

(23) *Oxytetracycline.* The term ''oxytetracycline master standard'' means a specific lot of oxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the oxytetracycline working standard.

(24) *Paromomycin.* The term ''paromomycin master standard'' means a specific lot of paromomycin designated by the Commissioner as the standard of comparison in determining the potency of the paromomycin working standard.

(25) *Polymyxin B.* The term ''polymyxin B master standard'' means a specific lot of polymyxin B designated by the Commissioner as the standard of comparison in determining the potency of the polymyxin B working standard.

(26) [Reserved]

(27) *Vancomycin.* The term ''vancomycin master standard'' means a specific lot of vancomycin designated by the Commissioner as the standard of comparison in determining the potency of the vancomycin working standard.

(28) [Reserved]

(29) *Troleandomycin.* The term ''troleandomycin master standard'' means a specific lot of troleandomycin designated by the Commissioner as the standard of comparison in determiing the potency of the troleandomycin working standard.

(30) *Gentamicin.* The term ''gentamicin master standard'' means a specific lot of gentamicin designated by the Commissioner as the standard of comparison in determining the potency of the gentamicin working standard.

(31) *Dactinomycin.* The term ''dactinomycin master standard'' means a specific lot of dactinomycin designated by the Commissioner as the standard of comparison in determining the potency of the dactinomycin working standard.

(32) *Candicidin.* The term ''candicidin master standard'' means a specific lot of candicidin that is designated by the Commissioner as the standard of comparison in determining the potency of the candicidin working standard.

(33) *Cephalothin.* The term ''cephalothin master standard'' means a specific lot of cephalothin designated by the Commissioner as the standard of comparison in determining the potency of the cephalothin working standard.

(34) *Lincomycin.* The term ''lincomycin master standard'' means a specific lot of lincomycin designated by the Commissioner as the standard of comparison in determining the potency of the lincomycin working standard.

(35) *Methacycline.* The term ''methacycline master standard'' means a specific lot of methacycline designated by the Commissioner as the standard of comparison in determining the potency of the methacycline working standard.

(36) *Doxycycline.* The term ''doxycycline master standard'' means a specific lot of α-6-deoxyoxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the doxycycline working standard.

(37) *Cephaloridine.* The term ''cephaloridine master standard'' means a specific lot of cephaloridine that is designated by the Commissioner as the standard of comparison in determining the potency of the cephaloridine working standard.

(38) *Plicamycin.* The term ''plicamycin master standard'' means a specific lot of plicamycin designated by the Commissioner as the standard of comparison in determining the potency of the plicamycin working standard.

(39) *Clindamycin.* The term ''clindamycin master standard'' means a specific lot of clindamycin designated by the Commissioner as the standard of comparison in determining the potency of the clindamycin working standard.

(40) *Cephaloglycin.* The term ''cephaloglycin master standard'' means a specific lot of cephaloglycin designated by the Commissioner as the standard of comparison in determining the potency of the cephaloglycin working standard.

(41) *Carbenicillin.* The term ''carbenicillin master standard'' means a specific lot of carbenicillin designated by the Commissioner as the standard of comparison in determining the potency of the carbenicillin working standard.

(42) *Cephalexin.* The term ''cephalexin master standard'' means a specific lot of cephalexin that is designated by the Commissioner as the standard of comparison in determining the potency of the cephalexin working standard.

(43) [Reserved]

(44) *Capreomycin.* The term ''capreomycin master standard'' means a specific lot of capreomycin designated by the Commissioner as the standard of comparison in determining the potency of the capreomycin working standard.

(45) *Rifampin.* The term ''rifampin master standard'' means a specific lot of rifampin designated by the Commissioner as the standard of comparison in determining the potency of the rifampin working standard.

(46) *Minocycline.* The term ''minocycline master standard'' means a specific lot of minocycline designated by the Commissioner as the standard of comparison in determining the potency of the minocycline working standard.

(47) *Spectinomycin.* The term ''spectinomycin master standard'' means a specific lot of spectinomycin designated by the Commissioner as the standard of comparison in determining the potency of the spectinomycin working standard.

(48) *Clindamycin palmitate hydrochloride.* The term ''clindamycin palmitate hydrochloride master standard'' means a specific lot of clindamycin palmitate hydrochloride designated by the Commissioner as the standard of comparison in determining the potency of the clindamycin palmitate hydrochloride working standard.

(49) *Carbenicillin indanyl.* The term ''carbenicillin indanyl master standard'' means a specific lot of carbenicillin indanyl designated by the Commissioner as the standard of comparison in determining the potency of the carbenicillin indanyl working standard.

(50) *Cephapirin.* The term ''cephapirin master standard'' means a specific lot of cephapirin that is designated by the Commissioner as the standard of comparison in determining the potency of the cephapirin working standard.

(51) *Cefazolin.* The term ''cefazolin master standard'' means a specific lot

of cefazolin that is designated by the Commissioner as the standard of comparison in determining the potency of the cefazolin working standard.

(52) *Mitomycin.* The term ''mitomycin master standard'' means a specific lot of crystalline mitomycin that is designated by the Commissioner as the standard of comparison in determining the potency of the mitomycin working standard.

(53) *Amoxicillin.* The term ''amoxicillin master standard'' means a specific lot of amoxicillin that is designated by the Commissioner as the standard of comparison in determining the potency of the amoxicillin working standard.

(54) [Reserved]

(55) *Cephradine.* The term ''cephradine master standard'' means a specific lot of cephradine that is designated by the Commissioner as the standard of comparison in determining the potency of the cephradine working standard.

(56) *Doxorubicin.* The term ''doxorubicin master standard'' means a specific lot of crystalline doxorubicin that is designated by the Commissioner as the standard of comparison in determining the potency of the doxorubicin working standard.

(57) *Bleomycin.* The term ''bleomycin master standard'' means a specific lot of bleomycin designated by the Commissioner as the standard of comparison in determining the potency of the bleomycin working standard.

(58) *Tobramycin.* The term ''tobramycin master standard'' means a specific lot of tobramycin designated by the Commissioner as the standard of comparison in determining the potency of the tobramycin working standard.

(59) *Amikacin.* The term ''amikacin master standard'' means a specific lot of amikacin designated by the Commissioner as the standard of comparison in determining the potency of the amikacin working standard.

(60) *Vidarabine.* The term ''vidarabine master standard'' means a specific lot of vidarabine that is designated by the Commissioner as the standard of comparison in determining the potency of the vidarabine working standard.

(61) *Ticarcillin.* The term ''ticarcillin master standard'' means a specific lot of ticarcillin designated by the Commissioner as the standard of comparison in determining the potency of the ticarcillin working standard.

(62) *Cefadroxil.* The term ''cefadroxil master standard'' means a specific lot of cefadroxil that is designated by the Commissioner as the standard of comparison in determining the potency of the cefadroxil working standard.

(63) *Natamycin.* The term ''natamycin master standard'' means a specific lot of natamycin designated by the Commissioner as the standard of comparison in determining the potency of the natamycin working standard.

(64) *Cefoxitin.* The term ''cefoxitin master standard'' means a specific lot of cefoxitin that is designated by the Commissioner as the standard of comparison in determining the potency of the cefoxitin working standard.

(65) *Cefamandole.* The term ''cefamandole master standard'' means a specific lot of cefamandole that is designated by the Commissioner as the standard of comparison in determining the potency of the cefamandole working standard.

(66) *Cefaclor.* The term ''cefaclor master standard'' means a specific lot of cefaclor that is designated by the Commissioner as the standard of comparison in determining the potency of the cefaclor working standard.

(67) *Cyclacillin.* The term ''cyclacillin master standard'' means a specific lot of cyclacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the cyclacillin working standard.

(68) *Daunorubicin.* The term ''daunorubicin master standard'' means a specific lot of daunorubicin that is designated by the Commissioner as the standard of comparison in determining the potency of the daunorubicin working standard.

(69) *Sisomicin.* The term ''sisomicin master standard'' means a specific lot of sisomicin that is designated by the Commissioner as the standard of comparison in determining the potency of the sisomicin working standard.

(70) *Meclocycline.* The term ''meclocycline master standard'' means a specific lot of meclocycline that is designated by the Commissioner as the standard of comparison in determining

the potency of the meclocycline working standard.

(71) *Cefotaxime.* The term ''cefotaxime master standard'' means a specific lot of cefotaxime that is designated by the Commissioner as the standard of comparison in determining the potency of the cefotaxime working standard.

(72) *Mezlocillin.* The term ''mezlocillin master standard'' means a specific lot of mezlocillin that is designated by the Commissioner as the standard of comparison in determining the potency of the mezlocillin working standard.

(73) *Moxalactam.* The term ''moxalactam master standard'' means a specific lot of moxalactam that is designated by the Commissioner as the standard of comparison in determining the potency of the moxalactam working standard.

(74) *Piperacillin.* The term ''piperacillin master standard'' means a specific lot of piperacillin that is designated by the Commissioner as the standard of comparison in determining the potency of the piperacillin working standard.

(75) *Azlocillin.* The term ''azlocillin master standard'' means a specific lot of azlocillin that is designated by the Commissioner as the standard of comparison in determining the potency of the azlocillin working standard.

(76) *Cefoperazone.* The term ''cefoperazone master standard'' means a specific lot of cefoperazone that is designated by the Commissioner as the standard of comparison in determining the potency of the cefoperazone working standard.

(77) *Netilmicin.* The term ''netilmicin master standard'' means a specific lot of netilmicin that is designated by the Commissioner as the standard of comparison in determining the potency of the netilmicin working standard.

(78) *Cefuroxime.* The term ''cefuroxime master standard'' means a specific lot of cefuroxime that is designated by the Commissioner as the standard of comparison in determining the potency of the cefuroxime working standard.

(79) *Ceftizoxime.* The term ''ceftizoxime master standard'' means a specific lot of ceftizoxime that is designated by the Commissioner as the standard of comparison in determining the potency of the ceftizoxime working standard.

(80) *Cyclosporine.* The term ''cyclosporine master standard'' means a specific lot of cyclosporine that is designated by the Commissioner as the standard of comparison in determining the potency of the cyclosporine working standard.

(81) *Ceforanide.* The term ''ceforanide master standard'' means a specific lot of ceforanide that is designated by the Commissioner as the standard of comparison in determining the potency of the ceforanide working standard.

(82) *Cefonicid.* The term ''cefonicid master standard'' means a specific lot of cefonicid that is designated by the Commissioner as the standard of comparison in determining the potency of the cefonicid working standard.

(83) *Clavulanic acid.* The term ''clavulanic acid master standard'' means a specific lot of clavulanic acid or a salt thereof that is designated by the Commissioner as the standard of comparison in determining the potency of the clavulanic acid working standard.

(84) *Amdinocillin.* The term ''amdinocillin master standard'' means a specific lot of amdinocillin that is designated by the Commissioner as the standard of comparison in determining the potency of the amdinocillin working standard.

(85) *Ceftriaxone.* The term ''ceftriaxone master standard'' means a specific lot of ceftriaxone that is designated by the Commissioner as the standard of comparison in determining the potency of the ceftriaxone working standard.

(86) *Ceftazidime.* The term ''ceftazidime master standard'' means a specific lot of ceftazidime that is designated by the Commissioner as the standard of comparison in determining the potency of the ceftazidime working standard.

(87) *Imipenem.* The term ''imipenem master standard'' means a specific lot of imipenem that is designated by the Commissioner as the standard of comparison in determining the potency of the imipenem working standard.

(88) *Cefotetan.* The term ''cefotetan master standard'' means a specific lot of cefotetan that is designated by the

Commissioner as the standard of comparison in determining the potency of the cefotetan working standard.

(89) *Aztreonam.* The term ''aztreonam master standard'' means a specific lot of aztreonam that is designated by the Commissioner as the standard of comparison in determining the potency of the aztreonam working standard.

(90) *Sulbactam.* The term ''sulbactam master standard'' means a specific lot of sulbactam that is designated by the Commissioner as the standard of comparison in determining the potency of the sulbactam working standard.

(91) *Cefuroxime axetil.* The term ''cefuroxime axetil master standard'' means a specific lot of cefuroxime axetil that is designated by the Commissioner as the standard of comparison in determining the potency of the cefuroxime axetil working standard.

(92) *Cefmenoxime.* The term ''cefmenoxime master standard'' means a specific lot of cefmenoxime that is designated by the Commissioner as the standard of comparison in determining the potency of the cefmenoxime working standard.

(93) *Cefixime.* The term ''cefixime master standard'' means a specific lot of cefixime that is designated by the Commissioner as the standard of comparison in determining the potency of the cefixime working standard.

(94) *Cefotiam.* The term ''cefotiam master standard'' means a specific lot of cefotiam that is designed by the Commissioner as the standard of comparison in determining the potency of the cefotiam working standard.

(95) *Clindamycin phosphate.* The term ''clindamycin phosphate master standard'' means a specific lot of clindamycin phosphate that is designed by the Commissioner as the standard for comparison in determining the potency of the clindamycin phosphate standard.

(96) *Mupirocin.* The term ''mupirocin master standard'' means a specific lot of mupirocin or a salt thereof that is designated by the Commissioner as the standard of comparison in determining the potency of the mupirocin working standard.

(97) *Cefmetazole.* The term ''cefmetazole master standard'' means a specific lot of cefmetazole that is designated by the Commissioner as the standard of comparison in determining the potency of the cefmetazole working standard.

(98) *Cefpiramide.* The term ''cefpiramide master standard'' means a specific lot of cefpiramide that is designated by the Commissioner as the standard of comparison in determining the potency of the cefpiramide working standard.

(99) *Clarithromycin.* The term ''clarithromycin master standard'' means a specific lot of clarithromycin that is designated by the Commissioner as the standard of comparison in determining the potency of the clarithromycin working standard.

(100) *Azithromycin.* The term ''azithromycin master standard'' means a specific lot of azithromycin that is designated by the Commissioner as the standard of comparison in determining the potency of the azithromycin working standard.

(101) *Cefprozil.* The term ''cefprozil master standard'' means a specific lot of the (Z) isomer of cefprozil and a specific lot of the (E) isomer of cefprozil that are designated by the Commissioner as the standard of comparison in determining the potency of the cefprozil working standard.

(102) *Idarubicin.* The term ''idarubicin master standard'' means a specific lot of idarubicin that is designated by the Commissioner as the standard of comparison in determining the potency of the idarubicin working standard.

(103) *Loracarbef.* The term ''loracarbef master standard'' means a specific lot of loracarbef that is designated by the Commissioner as the standard of comparison in determining the potency of the loracarbef working standard.

(104) *Rifabutin.* The term ''rifabutin master standard'' means a specific lot of rifabutin that is designated by the Commissioner as the standard of comparison in determining the potency of the rifabutin working standard.

(105) *Cefpodoxime proxetil.* The term ''cefpodoxime proxetil master standard'' means a specific lot of the (R) isomer of cefpodoxime proxetil that is designated by the Commissioner as the standard of comparison in determining the potency of the cefpodoxime proxetil working standard.

(b) *Working standards.* The potency or purity of each preparation has been determined by comparison with its master standard , and each has been designated by the Commissioner as working standards for use in determining the potency or purity of antibiotic substances subject to the regulations in this chapter.Unless otherwise noted, the working standard and the U.S.P. reference standard for the antibiotic drug named are identical.

(1) *Penicillin.* (i) The term ''penicillin G working standard'' means a specific lot of a homogeneous preparation of penicillin G.

(ii) [Reserved]

(iii) The term ''penicillin V working standard'' means a specific lot of a homogeneous preparation of penicillin V.

(iv) [Reserved]

(v) The term ''methicillin working standard'' means a specific lot of a homogeneous preparation of methicillin.

(vi) The term ''oxacillin working standard'' means a specific lot of a homogeneous preparation of oxacillin.

(vii) The term ''ampicillin working standard'' means a specific lot of a homogeneous preparation of ampicillin.

(viii) The term ''nafcillin working standard'' means a specific lot of a homogeneous preparation of nafcillin.

(ix) The term ''cloxacillin working standard'' means a specific lot of a homogeneous preparation of cloxacillin.

(x) The term ''penicillin G procaine working standard'' means a specific lot of a homogeneous preparation of penicillin G procaine.

(xi) The term ''dicloxacillin working standard'' means a specific lot of a homogeneous preparation of dicloxacillin.

(xii) The term ''bacampicillin hydrochloride working standard'' means a specific lot of a homogeneous preparation of bacampicillin hydrochloride.

(2) *Amphotericin A.* The term ''amphotericin A working standard'' means a specific lot of a homogeneous preparation of amphotericin A.

(3) *Amphotericin B.* The term ''amphotericin B working standard'' means a specific lot of a homogeneous preparation of amphotericin B.

(4) *Streptomycin.* The term ''streptomycin working standard'' means a specific lot of a homogeneous preparation of streptomycin.

(5) *Dihydrostreptomycin.* The term ''dihydrostreptomycin working standard'' means a specific lot of a homogeneous preparation of dihydrostreptomycin.

(6) *Chlortetracycline.* The term ''chlortetracycline working standard'' means a specific lot of a homogeneous preparation of chlortetracycline.

(7) *Demeclocycline.* The term ''demeclocycline working standard'' means a specific lot of a homogeneous preparation of demeclocycline.

(8) *Tetracycline.* The term ''tetracycline working standard'' means a specific lot of a homogeneous preparation of tetracycline.

(9) *Rolitetracycline.* The term ''rolitetracycline working standard'' means a specific lot of a homogeneous preparation of rolitetracycline.

(10) *Chloramphenicol.* The term ''chloramphenicol working standard'' means a specific lot of a homogeneous preparation of chloramphenicol.

(11) *Bacitracin.* The term ''bacitracin working standard'' means a specific lot of a homogeneous preparation of bacitracin.

(12) [Reserved]

(13) *Colistin.* The term ''colistin working standard'' means a specific lot of a homogeneous preparation of colistin.

(14) *Colistimethate.* The term ''colistimethate working standard'' means a specific lot of a homogeneous preparation of colistimethate.

(15) *Cycloserine.* The term ''cycloserine working standard'' means a specific lot of a homogeneous preparation of cycloserine.

(16) *Erythromycin.* The term ''erythromycin working standard'' means a specific lot of a homogeneous preparation of erythromycin.

(17) *Gramicidin.* The term ''gramicidin working standard'' means a specific lot of a homogeneous preparation of gramicidin.

(18) *Griseofulvin.* The term ''griseofulvin working standard'' means a specific lot of a homogeneous preparation of griseofulvin.

(19) *Kanamycin.* The term ''kanamycin working standard'' means a specific lot of a homogeneous preparation of kanamycin.

(20) *Neomycin.* The term ''neomycin working standard'' means a specific lot

of a homogeneous preparation of neomycin.

(21) *Novobiocin.* The term ''novobiocin working standard'' means a specific lot of a homogeneous preparation of novobiocin.

(22) *Nystatin.* The term ''nystatin working standard'' means a specific lot of a homogeneous preparation of nystatin.

(23) *Oleandomycin.* The term ''oleandomycin working standard'' means a specific lot of a homogeneous preparation of oleandomycin.

(24) *Troleandomycin.* The term ''troleandomycin working standard''means a specific lot of a homogeneous preparation of troleandomycin.

(25) *Oxytetracycline.* The term ''oxytetracycline working standard'' means a specific lot of a homogeneous preparation of oxytetracycline.

(26) *Paromomycin.* The term ''paromomycin working standard'' means a specific lot of a homogeneous preparation of paromomycin.

(27) *Polymyxin B.* The term ''polymyxin B working standard'' means a specific lot of a homogeneous preparation of polymyxin B.

(28) *Vancomycin.* The term ''vancomycin working standard'' means a specific lot of a homogeneous preparation of vancomycin.

(29) [Reserved]

(30) *Gentamicin.* The term ''gentamicin working standard'' means a specific lot of a homogeneous preparation of gentamicin.

(31) *Dactinomycin.* The term ''dactinomycin working standard'' means a specific lot of a homogeneous preparation of dactinomycin.

(32) *Candicidin.* The term ''candicidin working standard'' means a specific lot of a homogeneous preparation of candicidin.

(33) *Cephalothin.* The term ''cephalothin working standard'' means a specific lot of a homogeneous preparation of cephalothin.

(34) *Lincomycin.* The term ''lincomycin working standard'' means a specific lot of a homogeneous preparation of lincomycin.

(35) *Methacycline.* The term ''methacycline working standard'' means a specific lot of homogeneous preparation of methacycline.

(36) *Doxycycline.* The term ''doxycycline working standard'' means a specific lot of homogeneous preparation of α-6-deoxyoxytetracycline.

(37) *Cephaloridine.* The term ''cephaloridine working standard'' means a specific lot of homogeneous preparation of cephaloridine.

(38) *Plicamycin.* The term ''plicamycin working standard'' means a specific lot of a homogeneous preparation of plicamycin.

(39) *Clindamycin.* The term ''clindamycin working standard'' means a specific lot of a homogeneous preparation of clindamycin.

(40) *Cephaloglycin.* The term ''cephaloglycin working standard'' means a specific lot of homogeneous preparation of cephaloglycin.

(41) *Carbenicillin.* The term ''carbenicillin working standard'' means a specific lot of homogeneous preparation of carbenicillin.

(42) *Cephalexin.* The term ''cephalexin working standard'' means a specific lot of a homogeneous preparation of cephalexin.

(43) [Reserved]

(44) *Capreomycin.* The term ''capreomycin working standard'' means a specific lot of a homogeneous preparation of capreomycin.

(45) *Rifampin.* The term ''rifampin working standard'' means a specific lot of a homogeneous preparation of rifampin.

(46) *Minocycline.* The term ''minocycline working standard'' means a specific lot of a homogeneous preparation of minocycline.

(47) *Spectinomycin.* The term ''spectinomycin working standard'' means a specific lot of a homogeneous preparation of spectinomycin.

(48) *Clindamycin palmitate hydrochloride.* The term ''clindamycin palmitate hydrochloride working standard'' means a specific lot of a homogeneous preparation of clindamycin palmitate hydrochloride.

(49) *Carbenicillin indanyl.* The term ''carbenicillin indanyl working standard'' means a specific lot of a homogeneous preparation of carbenicillin indanyl.

(50) *Cephapirin.* The term ''cephapirin working standard'' means a specific lot of a homogeneous preparation of cephapirin.

(51) *Cefazolin.* The term ''cefazolin working standard'' means a specific lot of a homogeneous preparation of cefazolin.

(52) *Mitomycin.* The term ''mitomycin working standard'' means a specific lot of a homogeneous preparation of mitomycin.

(53) *Amoxicillin.* The term ''amoxicillin working standard'' means a specific lot of a homogeneous preparation of amoxicillin.

(54) [Reserved]

(55) *Cephradine.* The term ''cephradine working standard'' means a specific lot of a homogeneous preparation of cephradine.

(56) *Doxorubicin.* The term ''doxorubicin working standard'' means a specific lot of a homogeneous preparation of doxorubicin.

(57) *Bleomycin.* The term ''bleomycin working standard'' means a specific lot of a homogeneous preparation of bleomycin.

(58) *Tobramycin.* The term ''tobramycin working standard'' means a specific lot of a homogeneous preparation of tobramycin.

(59) *Amikacin.* The term ''amikacin working standard'' means a specific lot of a homogeneous preparation of amikacin.

(60) *Vidarabine.* The term ''vidarabine working standard'' means a specific lot of a homogeneous preparation of vidarabine.

(61) *Ticarcillin.* The term ''ticarcillin working standard'' means a specific lot of a homogeneous preparation of ticarcillin.

(62) *Cefadroxil.* The term ''cefadroxil working standard'' means a specific lot of a homogeneous preparation of cefadroxil.

(63) *Natamycin.* The term ''natamycin working standard'' means a specific lot of a homogeneous preparation of natamycin.

(64) *Cefoxitin.* The term ''cefoxitin working standard'' means a specific lot of a homogeneous preparation of cefoxitin.

(65) *Cefamandole.* The term ''cefamandole working standard'' means a specific lot of a homogeneous preparation of cefamandole.

(66) *Cefaclor.* The term ''cefaclor working standard'' means a specific lot of a homogeneous preparation of cefaclor.

(67) *Cyclacillin.* The term ''cyclacillin working standard'' means a specific lot of a homogeneous preparation of cyclacillin.

(68) *Daunorubicin.* The term ''daunorubicin working standard'' means a specific lot of a homogeneous preparation of daunorubicin.

(69) *Sisomicin.* The term ''sisomicin working standard'' means a specific lot of a homogeneous preparation of sisomicin.

(70) *Meclocycline.* The term ''meclocycline working standard'' means a specific lot of a homogeneous preparation of meclocycline.

(71) *Cefotaxime.* The term ''cefotaxime working standard'' means a specific lot of a homogeneous preparation of cefotaxime.

(72) *Mezlocillin.* The term ''mezlocillin working standard'' means a specific lot of a homogeneous preparation of mezlocillin.

(73) *Moxalactam.* The term ''moxalactam working standard'' means a specific lot of a homogeneous preparation of moxalactam.

(74) *Piperacillin.* The term ''piperacillin working standard'' means a specific lot of a homogeneous preparation of piperacillin.

(75) *Azlocillin.* The term ''azlocillin working standard'' means a specific lot of a homogeneous preparation of azlocillin.

(76) *Cefoperazone.* The term ''cefoperazone working standard'' means a specific lot of a homogeneous preparation of cefoperazone.

(77) *Netilmicin.* The term ''netimicin working standard'' means a specific lot of a homogeneous preparation of netilmicin.

(78) *Cefuroxime.* The term ''cefuroxime working standard'' means a specific lot of a homogeneous preparation of cefuroxime.

(79) *Ceftizoxe.* The term ''ceftizoxime working standard'' means a specific lot of a homogeneous preparation of ceftizoxime.

(80) *4-Epitetracycline.* The term ''4-epitetracycline working standard'' means a specific lot of a homogeneous preparation of 4-epitetracycline.

(81) *Chloramphenicol palmitate.* The term ''chloramphenicol palmitate working standard'' means a specific lot of a homogeneous preparation of chloramphenicol palmitate.

(82) *Cyclosporine.* The term ''cyclosporine working standard'' means a specific lot of a homogeneous preparation of cyclosporine.

(83) *Ceforanide.* The term ''ceforanide working standard'' means a specific lot of a homogeneous preparation of ceforanide.

(84) *Cefonicid.* The term ''cefonicid working standard'' means a specific lot of a homogeneous preparation of cefonicid.

(85) *Clavulanic acid.* The term ''clavulanic acid working standard'' means a specific lot of a homogeneous preparation of clavulanic acid or a salt thereof.

(86) *Amdinocillin.* The term ''amdinocillin working standard'' means a specific lot of a homogeneous preparation of amdinocillin.

(87) *Ceftriaxone.* The term ''ceftriaxone working standard'' means a specific lot of a homogeneous preparation of ceftriaxone.

(88) *Ceftazidime.* The term ''ceftazidime working standard'' means a specific lot of a homogeneous preparation of ceftazidime.

(89) *Imipenem.* The term ''imipenem working standard'' means a specific lot of a homogeneous preparation of imipenem.

(90) *Cefotetan.* The term ''cefotetan working standard'' means a specific lot of a homogeneous preparation of cefotetan.

(91) *Aztreonam.* The term ''aztreonam working standard'' means a specific lot of a homogeneous preparation of aztreonam.

(92) *Sulbactam.* The term ''sulbactam working standard'' means a specific lot of a homogeneous preparation of sulbactam.

(93) *Cefuroxime axetil.* The term ''cefuroxime axetil working standard'' means a specific lot of a homogeneous preparation of cefuroxime axetil.

(94) *Cefmenoxime.* The term ''cefmenoxime working standard'' means a specific lot of a homogeneous preparation of cefmenoxime.

(95) *Cefixime.* The term ''cefixime working standard'' means a specific lot of a homogeneous preparation of cefixime.

(96) *Cefotiam.* The term ''cefotiam working standard'' means a specific lot of a homogenous preparation of cefotiam.

(97) *Clindamycin phosphate.* The term ''clindamycin phosphate working standard'' means a specific lot of a homogenous preparation of clindamycin phosphate.

(98) *Mupirocin.* The term ''mupirocin working standard'' means a specific lot of a homogeneous preparation of mupirocin or a salt thereof.

(99) *Cefmetazole.* The term ''cefmetazole working standard'' means a specific lot of a homogeneous preparation of cefmetazole.

(100) *Cefpiramide.* The term ''cefpiramide working standard'' means a specific lot of a homogeneous preparation of cefpiramide.

(101) *Clarithromycin.* The term ''clarithromycin working standard'' means a specific lot of a homogeneous preparation of clarithromycin.

(102) *Azithromycin.* The term ''azithromycin working standard'' means a specific lot of a homogeneous preparation of azithromycin.

(103) *Cefprozil.* The term ''cefprozil (Z) working standard'' means a specific lot of a homogeneous preparation of cefprozil (Z). The term ''cefprozil (E) working standard'' means a specific lot of a homogeneous preparation of cefprozil (E).

(104) *Idarubicin.* The term ''idarubicin working standard'' means a specific lot of a homogeneous preparation of idarubicin.

(105) *Loracarbef.* The term ''loracarbef working standard'' means a specific lot of a homogeneous preparation of loracarbef.

(106) *Rifabutin.* The term ''rifabutin working standard'' means a specific lot of a homogeneous preparation of rifabutin.

(107) *Cefpodoxime proxetil.* The term ''cefpodoxime proxetil working standard'' means a specific lot of a homogeneous preparation of cefpodoxime proxetil.

[39 FR 18925, May 30, 1974]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 430.5, see the List of CFR Sections Affected appearing in the Finding Aids section of this volume.

§ 430.6 Definitions of the terms "unit" and "microgram" as applied to antibiotic substances.

Unless it has been otherwise specified in the individual definitions in this section, the activity assigned to each ''unit'' or ''microgram'' is equivalent to an International Unit, if such has been defined by the World Health Organization.

(a) *''Unit''*—(1) *Penicillin*—(i) *Penicillin G.* The term ''unit'' applies to penicillin G means the penicillin activity (potency) contained in 0.600 microgram of the penicillin G master standard.

(ii) [Reserved]

(iii) *Penicillin V.* The term ''unit'' applied to penicillin V means the penicillin activity (potency) contained in 0.590 microgram of the penicillin V master standard.

(2) *Bacitracin.* The term ''unit'' applied to bacitracin means a bacitracin activity (potency) contained in 13.51 micrograms of the bacitracin master standard, except that when the activity (potency) of bacitracin is expressed in terms of its weight, as in the feed and drinking water of animals, 1 gram of activity is equivalent to 42,000 units.

(3) *Nystatin.* The term ''unit'' applied to nystatin means the nystatin activity (potency) contained in 0.2817 microgram of the nystatin master standard when dried for 2 hours at 40° C. and a pressure of 5 millimeters or less.

(4) *Polymyxin B.* The term ''unit'' applied to polymyxin B means the polymyxin activity (potency) contained in 0.1274 microgram of the polymyxin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(5) *Bleomycin.* The term ''unit'' applied to bleomycin means the bleomycin activity (potency) contained in 0.637 milligram of the bleomycin master standard.

(b) *''Microgram''*—(1) *Streptomycin.* The term ''microgram'' applied to streptomycin means the streptomycin activity (potency) contained in 1.250 micrograms of the streptomycin master standard after it is dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(2) *Dihydrostreptomycin.* The term ''microgram'' applied to dihydrostreptomycin means the dihydrostreptomycin activity (potency) contained in 1.25 micrograms of the dihydrostreptomycin master standard after it is dried for 4 hours at 100° C. and a pressure of 50 microns or less.

(3) *Chlortetracycline.* The term ''microgram'' applied to chlortetracycline means the chlortetracycline activity (potency) contained in 1.0 microgram of the chlortetracycline master standard.

(4) *Demeclocycline.* The term ''microgram'' applied to demeclocycline means the demeclocycline activity (potency) contained in 1.0 microgram of the demeclocycline master standard after it is dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(5) *Tetracycline.* The term ''microgram'' applied to tetracycline means the tetracycline activity (potency) contained in 1.0 microgram of tetracycline master standard.

(6) *Rolitetracycline.* The term ''microgram'' applied to rolitetracycline means the rolitetracycline activity (potency) contained in 1.0 microgram of the rolitetracycline master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(7) *Chloramphenicol.* The term ''microgram'' applied to chloramphenicol means the chloramphenicol activity (potency) contained in 1.0 microgram of the chloramphenicol master standard.

(8) *Methicillin.* The term ''microgram'' applied to methicillin means the methicillin activity (potency) contained in 1.105 micrograms of the methicillin master standard.

(9) *Oxacillin.* The term ''microgram'' applied to oxacillin means the oxacillin activity (potency) contained in 1.111

micrograms of the oxacillin master standard.

(10) [Reserved]

(11) *Amphotericin A.* The term ''microgram'' applied to amphotericin A means the amphotericin A activity (potency) contained in 1.0 microgram of the amphotericin A master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(12) *Amphotericin B.* The term ''microgram'' applied to amphotericin B means the amphotericin B activity (potency) contained in 1.014 micrograms of the amphotericin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(13) *Colistin.* The term ''microgram'' applied to colistin means the colistin base activity (potency) contained in 1.495 micrograms of the colistin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less. The numerical value of a microgram of colistin is not equivalent to the International Unit.

(14) *Colistimethate.* The term ''microgram'' applied to colistimethate means the activity (potency) calculated as colistin base that is contained in 1.938 micrograms of the colistimethate master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less. The numerical value of a microgram of colistimethate is not equivalent to the International Unit.

(15) *Cycloserine.* The term ''microgram'' applied to cycloserine means the cycloserine activity (potency) contained in 1.0 microgram of the cycloserine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(16) *Erythromycin.* The term ''microgram'' applied to erythromycin means the erythromycin base activity (potency) contained in 1.02 micrograms of the erythromycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(17) *Gramicidin.* The term ''microgram'' applied to gramicidin means the gramicidin activity (potency) contained in 1.0 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(18) *Griseofulvin.* The term ''microgram'' applied to griseofulvin means the griseofulvin activity (potency) contained in 1.0 microgram of the griseofulvin master standard.

(19) *Kanamycin.* The term ''microgram'' applied to kanamycin means the kanamycin base activity (potency) contained in 1.299 micrograms of the kanamycin master standard.

(20) *Neomycin.* The term ''microgram'' applied to neomycin means the neomycin base activity (potency) contained in 1.429 micrograms of the neomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(21) *Novobiocin.* The term ''microgram'' applied to novobiocin means the novobiocin acid activity (potency) contained in 1.033 micrograms of the novobiocin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(22) *Oleandomycin.* The term ''microgram'' applied to oleandomycin means the oleandomycin base activity (potency) contained in 1.176 micrograms of the oleandomycin master standard.

(23) *Troleandomycin.* The term ''microgram'' applied to troleandomycin means the activity (potency), calculated as the molecular equivalent of the oleandomycin base, contained in 1.2315 micrograms of the troleandomycin master standard.

(24) *Oxytetracycline.* The ''microgram'' applied to oxytetracycline means the oxytetracycline base activity (potency) contained in 1.13 micrograms of the oxytetracycline master standard.

(25) *Paromomycin.* The term ''microgram'' applied to paromomycin means the paromomycin activity (potency) contained in 1.333 micrograms of the paromomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(26) *Tyrothricin.* The term ''microgram'' applied to tyrothricin means the activity (potency) contained in 0.2 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(27) *Vancomycin.* The term ''microgram'' applied to vancomycin means the vancomycin base activity (potency) contained in 1.25 micrograms of the vancomycin master standard.

(28) [Reserved]

(29) *Ampicillin.* The term ''microgram'' applied to ampicillin means the ampicillin activity (potency) contained in 1.1764 micrograms of the ampicillin master standard.

(30) *Nafcillin.* The term ''microgram'' applied to nafcillin means the nafcillin activity (potency) contained in 1.0989 micrograms of the nafcillin master standard.

(31) *Gentamicin.* The term ''microgram'' applied to gentamicin means the gentamicin activity (potency) contained in 1.56 micrograms of the gentamicin master standard when dried for 3 hours at 110° C. and a pressure of 5 millimeters or less.

(32) *Dactinomycin.* The term ''microgram'' applied to dactinomycin means the dactinomycin activity (potency) contained in 1.000 microgram of the dactinomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(33) *Candicidin.* The term ''microgram'' applied to candicidin means the candicidin activity (potency) contained in 1.0 microgram of the candicidin master standard when dried for 3 hours at 40° C. and a pressure of 5 millimeters or less.

(34) *Cephalothin.* The term ''microgram'' applied to cephalothin means the cephalothin activity (potency) contained in 1.056 micrograms of the cephalothin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(35) *Lincomycin.* The term ''microgram'' applied to lincomycin means the lincomycin base activity (potency) contained in 1.156 micrograms of the lincomycin master standard.

(36) *Cloxacillin.* The term ''microgram'' applied to cloxacillin means the cloxacillin activity (potency) contained in 1.135 micrograms of the cloxacillin master standard.

(37) *Methacycline.* The term ''microgram'' applied to methacycline means the methacycline activity (potency) contained in 1.082 micrograms of the methacycline master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(38) *Doxycycline.* The term ''microgram'' applied to doxycycline means the doxycycline activity (potency) contained in 1.155 micrograms of the doxycycline master standard.

(39) *Cephaloridine.* The term ''microgram'' applied to cephaloridine means the cephaloridine activity (potency) contained in 1.00806 micrograms of the cephaloridine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(40) *Dicloxacillin.* The term ''microgram'' applied to dicloxacillin means the dicloxacillin activity (potency) contained in 1.087 micrograms of the dicloxacillin master standard.

(41) *Plicamycin.* The term ''microgram'' applied to plicamycin means the plicamycin activity (potency) contained in 1.000 microgram of the plicamycin master standard when dried for 4 hours at 25° C. and a pressure of 5 millimeters or less.

(42) *Clindamycin.* The term ''microgram'' applied to clindamycin means the clindamycin activity (potency) contained in 1.139 micrograms of the clindamycin master standard.

(43) *Cephaloglycin.* The term ''microgram'' applied to cephaloglycin means the cephaloglycin activity (potency) contained in 1.02564 micrograms of the cephaloglycin master standard.

(44) *Carbenicillin.* The term ''microgram'' applied to carbenicillin means the carbenicillin activity (potency) contained in 1.135 micrograms of the carbenicillin master standard.

(45) *Cephalexin.* The term ''microgram'' applied to cephalexin means the cephalexin activity (potency) contained in 1.0707 micrograms of the cephalexin master standard.

(46) [Reserved]

(47) *Capreomycin.* The term ''microgram'' applied to capreomycin means the capreomycin activity (potency) contained in 1.0870 micrograms of the capreomycin master standard when dried for 4 hours at 100° C. and a pressure of 5 millimeters or less.

(48) *Rifampin.* The term ''microgram'' applied to rifampin means the rifampin activity (potency) contained in 1.0101

micrograms of the rifampin master standard.

(49) *Minocycline.* The term ''microgram'' applied to minocycline means the minocycline activity (potency) contained in 1.1588 micrograms of the minocycline master standard.

(50) *Spectinomycin.* The term ''microgram'' applied to spectinomycin means the spectinomycin activity (potency) contained in 1.490 micrograms of the spectinomycin master standard.

(51) *Clindamycin palmitate hydrochloride.* The term ''microgram'' applied to clindamycin palmitate hydrochloride means the clindamycin activity (potency) contained in 1.661 micrograms of the clindamycin palmitate hydrochloride master standard.

(52) *Carbenicillin indanyl.* The term ''microgram'' applied to carbenicillin indanyl means the carbenicillin activity (potency) contained in 1.4514 micrograms of the carbenicillin indanyl master standard.

(53) *Cephapirin.* The term ''microgram'' applied to cephapirin means the cephapirin activity (potency) contained in 1.0616 micrograms of the cephapirin master standard.

(54) *Cefazolin.* The term ''microgram'' applied to cefazolin means the cefazolin activity (potency) contained in 1.005 micrograms of the cefazolin master standard.

(55) *Mitomycin.* The term ''microgram'' applied to mitomycin means the mitomycin activity (potency) contained in 1.0416 micrograms of the mitomycin master standard.

(56) *Amoxicillin.* The term ''microgram'' applied to amoxicillin means the amoxicillin activity (potency) contained in 1.17647 micrograms of the amoxicillin master standard.

(57) [Reserved]

(58) *Cephradine.* The term ''microgram'' applied to cephradine means the cephradine activity (potency) contained in 1.1111 micrograms of the cephradine master standard.

(59) *Doxorubicin.* The term ''microgram'' applied to doxorubicin means the activity (potency) calculated as doxorubicin hydrochloride contained in 1.0204 micrograms of the doxorubicin master standard.

(60) *Tobramycin.* The term ''microgram'' applied to tobramycin means the tobramycin activity (potency) contained in 1.126 micrograms of the tobramycin master standard.

(61) *Amikacin.* The term ''microgram'' applied to amikacin means the amikacin activity (potency) contained in 1.091 micrograms of the amikacin master standard.

(62) *Vidarabine.* The term ''microgram'' applied to vidarabine means the vidarabine activity (potency) contained in 1.0674 micrograms of the vidarabine master standard.

(63) *Ticarcillin.* The term ''microgram'' applied to ticarcillin means the ticarcillin activity (potency) contained in 1.136 micrograms of the ticarcillin master standard.

(64) *Cefadroxil.* The term ''microgram'' applied to cefadroxil means the cefadroxil activity (potency) contained in 1.0537 micrograms of the cefadroxil master standard.

(65) *Natamycin.* The term ''microgram'' applied to natamycin means the natamycin activity (potency) contained in 1.0846 micrograms of the natamycin master standard.

(66) *Cefoxitin.* The term ''microgram'' applied to cefoxitin means the cefoxitin activity (potency) contained in 1.072 micrograms of the cefoxitin master standard.

(67) *Cefamandole.* The term ''microgram'' applied to cefamandole means the cefamandole activity (potency) contained in 1.1364 micrograms of cefamandole master standard.

(68) *Cefaclor.* The term ''microgram'' applied to cefaclor means the cefaclor activity (potency) contained in 1.0493 micrograms of cefaclor master standard.

(69) *Cyclacillin.* The term ''microgram'' applied to cyclacillin means the cyclacillin activity (potency) contained in 1.01 micrograms of the cyclacillin master standard.

(70) *Daunorubicin.* The term ''microgram'' applied to daunorubicin means the daunorubicin activity (potency) contained in 1.0965 micrograms of the daunorubicin master standard.

(71) *Sisomicin.* The term ''microgram'' applied to sisomicin means the sisomicin activity (potency) contained

in 1.00 microgram of the sisomicin master standard expressed on an anhydrous basis.

(72) *Meclocycline.* The term ''microgram'' applied to meclocycline means the meclocycline activity (potency) contained in 1.0493 micrograms of the meclocycline master standard.

(73) *Cefotaxime.* The term ''microgram'' applied to cefotaxime means the cefotaxime activity (potency) contained in 1.089 micrograms of cefotaxime master standard.

(74) *Mezlocillin.* The term ''microgram'' applied to mezlocillin means the mezlocillin activity (potency) contained in 1.1086 micrograms of the mezlocillin master standard.

(75) *Moxalactam.* The term ''microgram'' applied to moxalactam means the moxalactam activity (potency) contained in 1.1173 micrograms of the moxalactam master standard.

(76) *Piperacillin.* The term ''microgram'' applied to piperacillin means the piperacillin activity (potency) contained in 1.0460 micrograms of the piperacillin master standard.

(77) *Cefoperazone.* The term ''microgram'' applied to cefoperazone means the cefoperazone activity (potency) contained in 1.056 micrograms of the cefoperazone master standard.

(78) *Azlocillin.* The term ''microgram'' applied to azlocillin means the azlocillin activity (potency) contained in 1.128 micrograms of the azlocillin master standard.

(79) *Netilmicin.* The term ''microgram'' applied to netilmicin means the netilmicin activity (potency) contained in 1.000 microgram of the netilmicin master standard expressed on an anhydrous basis.

(80) *Cefuroxime.* The term ''microgram'' applied to cefuroxime means the cefuroxime activity (potency) contained in 1.0893 micrograms of the cefuroxime master standard.

(81) *Ceftizoxime.* The term ''microgram'' applied to ceftizoxime means the ceftizoxime activity (potency) contained in 1.011 micrograms of the ceftizoxime master standard.

(82) *Cyclosporine.* The term ''microgram'' applied to cyclosporine means the cyclosporine activity (potency) contained in 1.0173 micrograms of cyclosporine master standard.

(83) *Ceforanide.* The term ''microgram'' applied to ceforanide means the ceforanide activity (potency) contained in 1.005 micrograms of the ceforanide master standard.

(84) *Cefonicid.* The term ''microgram'' applied to cefonicid means the cefonicid activity (potency) contained in 1.150 micrograms of the cefonicid master standard.

(85) *Clavulanic acid.* The term ''microgram'' applied to clavulanic acid means the clavulanic acid activity (potency) contained in 1.053 micrograms of clavulanic acid master standard.

(86) *Amdinocillin.* The term ''microgram'' applied to amdinocillin means the amdinocillin activity (potency) contained in 1.004 micrograms of the amdinocillin master standard.

(87) *Ceftriaxone.* The term ''microgram'' applied to ceftriaxone means the ceftriaxone activity (potency) contained in 1.19 micrograms of the ceftriaxone master standard.

(88) *Ceftazidime.* The term ''microgram'' applied to ceftazidime means the ceftazidime activity (potency) contained in 1.1834 micrograms of the ceftazidime master standard.

(89) *Imipenem.* The term ''microgram'' applied to imipenem monohydrate means the imipenem activity (potency) contained in 1.085 micrograms of the imipenem master standard.

(90) *Cefotetan.* The term ''microgram'' applied to cefotetan means the cefotetan activity (potency) contained in 1.012 micrograms of the cefotetan master standard.

(91) *Aztreonam.* The term ''microgram'' applied to aztreonam means the aztreonam activity (potency) contained in 1.05 micrograms of the aztreonam master standard.

(92) *Sulbactam.* The term ''microgram'' applied to sulbactam means the sulbactam activity (potency) contained in 1.002 micrograms of the sulbactam master standard.

(93) *Cefuroxime axetil.* The term ''microgram'' applied to cefuroxime axetil means the cefuroxime activity (potency) contained in 1.246 micrograms of the cefuroxime axetil master standard.

(94) *Cefmenoxime.* The term ''microgram'' applied to cefmenoxime

means the cefmenoxime activity (potency) contained in 1.0482 micrograms of the cefmenoxime master standard.

(95) *Cefixime.* The term ''microgram'' applied to cefixime means the cefixime activity (potency) contained in 1.126 micrograms of the cefixime master standard.

(96) *Cefotiam.* The term ''microgram'' applied to cefotiam means the cefotiam (potency) contained in 1.144 micrograms of the cefotiam master standard.

(97) *Clindamycin phosphate.* The term ''microgram'' applied to clindamycin phosphate means the clindamycin phosphate (potency) contained in 1.252 micrograms of the clindamycin phosphate master standard.

(98) *Mupirocin.* The term ''microgram'' applied to mupirocin means the activity (potency) calculated as mupirocin activity (potency) contained in 1.075 micrograms of the mupirocin master standard.

(99) *Cefmetazole.* The term ''microgram'' applied to cefmetazole means the cefmetazole (potency) contained in 1.002 micrograms of the cefmetazole master standard.

(100) *Cefpiramide.* The term ''microgram'' applied to cefpiramide means the cefpiramide (potency) contained in 0.994 microgram of the cefpiramide master standard.

(101) *Clarithromycin.* The term ''microgram'' applied to clarithromycin means the clarithromycin (potency) contained in 1.010 micrograms of the clarithromycin master standard.

(102) *Azithromycin.* The term ''microgram'' applied to azithromycin means the azithromycin (potency) contained in 1.063 micrograms of the azithromycin master standard.

(103) *Cefprozil.* The term ''microgram'' applied to cefprozil (Z) means the cefprozil (Z) potency contained in 1.060 micrograms of the cefprozil (Z) master standard. The term ''microgram'' applied to cefprozil (E) means the cefprozil (E) potency contained in 1.106 micrograms of the cefprozil (E) master standard.

(104) *Idarubicin.* The term ''microgram'' applied to idarubicin means the idarubicin activity (potency) calculated as idarubicin hydrochloride contained in 1.036 micrograms of the idarubicin master standard.

(105) *Loracarbef.* The term ''microgram'' applied to loracarbef means the loracarbef (potency) contained in 1.059 micrograms of the loracarbef master standard.

(106) *Rifabutin.* The term ''microgram'' applied to rifabutin means the rifabutin (potency) contained in 1.022 micrograms of the rifabutin master standard.

(107) *Cefpodoxime proxetil.* The term ''microgram'' applied to cefpodoxime proxetil means the cefpodoxime (potency) contained in 1.304 micrograms of the cefpodoxime proxetil master standard when dried.

[39 FR 18925, May 30, 1974]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 430.6, see the List of CFR Sections Affected appearing in the Finding Aids section of this volume.

Subpart B—Antibiotic Drugs Affected by the Drug Amendments of 1962

§ 430.10 Certification or release of antibiotic drugs affected by the drug amendments of 1962.

(a) Before the 1962 amendments to it, the Federal Food, Drug, and Cosmetic Act only permitted the Food and Drug Administration to provide for the certification of batches of antibiotic drugs containing penicillin, streptomycin, chlortetracycline, chloramphenicol, or bacitracin, or any derivative of them. FDA certified those drugs under regulations promulgated on the basis of scientific proof of the drugs' safety and effectiveness. Most drugs containing an antibiotic other than one of those listed were subject to the new drug provisions of the act, which required that an applicant show that the drug was safe and obtain FDA approval of a new drug application before marketing it. An affirmative showing of effectiveness was not then required to obtain approval. Some antibiotic drugs that were not subject to certification, however, were also not subject to the new drug provisions of the act under informal FDA opinions that the drug was ''not a new drug'' or ''no longer a new drug.'' FDA

revoked those opinions under §310.100 of this chapter.

(b) The 1962 amendments amended section 507 of the act to require the certification, release without certification, or exemption from certification, of all antibiotic drugs on the basis of scientific proof of safety and effectiveness. The amendments provided that FDA implement them for antibiotic drugs that were marketed on April 30, 1963 and were not subject to the certification provisions on that date. FDA is implementing the amendments with respect to antibiotic drugs formerly subject to the new drug provisions of the act through its Drug Efficacy Study Implementation (DESI) program under which the agency is evaluating those antibiotic drugs for efficacy. Until FDA completes that evaluation it will permit continued marketing of those antibiotic drugs under paragraph (c) of this section. The agency is also implementing the 1962 amendments with respect to antibiotic drugs formerly not subject to either the certification or new drug provisions of the act and the agency is evaluating those antibiotic drugs for both safety and efficacy. Until FDA completes that evaluation, it will permit continued marketing of those antibiotic drugs under paragraph (d) of this section.

(c) Unless exempted from certification, FDA will certify or release antibiotic drugs which on April 30, 1963 were the subject of an approved new drug application under section 505 of the act, under regulations providing for certification of the drugs. Although the initial regulation for each of these drugs established under section 507(h) of the act was not conditioned upon an affirmative finding of the effectiveness of the drug, FDA is proceeding under its DESI program to amend or repeal those regulations to provide for certification of those drugs only if they had been shown to be both safe and effective.

(d) Unless exempted from certification, FDA will release without certification an antibiotic drug that was marketed on April 30, 1963, but not subject to certification, and not subject to an approved new drug application on that date, unless FDA has made a determination that the drug has not been shown to be safe or lacks substantial evidence of effectiveness under the DESI program. FDA is proceeding under its DESI program to establish regulations under section 507 to provide for certification of those drugs only if they have been shown to be safe and effective.

[50 FR 7516, Feb. 22, 1985]

PART 431—CERTIFICATION OF ANTIBIOTIC DRUGS

Subpart A—General Provisions

Sec.
431.1 Requests for certification, check tests and assays, and working standards; information and samples required.
431.5 Samples for sterility testing.
431.10 Certification.
431.11 Conditions on the effectiveness of certificates.
431.12 Certification of antibiotic drugs after shipment in bulk containers.
431.17 Request to provide for certification of an antibiotic drug.
431.20 Disposition of outdated drugs.

Subpart B—Administrative Procedures

431.50 Forms for certification or exemption of antibiotic drugs.
431.51 Suspension of certification service.
431.52 Hearings.
431.53 Fees.

Subpart C—Records and Reports

431.61 Records of distribution.
431.62 Records retention.

Subpart D—Confidentiality of Information

431.70 Confidentiality of data and information in an investigational new drug notice for an antibiotic drug.

AUTHORITY: 21 U.S.C. 351, 352, 353, 355, 357, 379e; 42 U.S.C. 216, 241, 262; 5 U.S.C. 552.

SOURCE: 39 FR 18934, May 30, 1974, unless otherwise noted.

Subpart A—General Provisions

§431.1 Requests for certification, check tests and assays, and working standards; information and samples required.

(a) A request for certification of a batch (antibiotic Form 7/Form FDA–1677) is to be addressed to the Food and

Drug Administration, Division of Research and Testing (HFD–470), 200 C St. SW., Washington, DC 20204.

(b) [Reserved]

(c) A person who requests certification or check tests and assays of a batch shall submit with his request the following information and samples:

(1) The batch mark of the drug.

(2) The quantity of each ingredient used in making the batch and a statement that each such ingredient conforms to the requirements or standards prescribed therefor, if any, by specific regulations or official compendium or otherwise approved by the Commissioner.

(3) The size of the batch, including the number of containers of each size in the batch.

(4) The date of the latest assay of the batch.

(5) The results of the latest tests and assays made by or for him on the batch as required for the drug by specific regulations.

(6) The batch mark(s) of the antibiotic(s) used in making the batch.

(7) Unless previously submitted, the results and dates of the latest tests and assays made by or for him on the antibiotic(s) used in making the batch as required by specific regulations.

(8) The number of accurately representative samples that are required for the batch by specific regulations:

(i) In the case of drugs such as dry powders, solutions, ointments, and suspensions, the sample shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal. In no case, however, shall more than 5,000 immediate containers have been packaged during each such interval of sampling, except for a sample collected for sterility testing.

(ii) In the case of drugs in unit dosage forms, such as tablets, capsules, or suppositories, samples shall be collected as follows:

(*a*) From batches exceeding 500,000 units, a representative sample consisting of 100 units shall be collected by taking single units at approximately equal intervals throughout the final production of the batch. If the person packaging the units into dispensing-size containers is not the manufacturer, the representative sample consisting of 100 units shall be collected by taking single units at approximately equal intervals during packaging.

(*b*) From batches of 500,000 units or less, a representative sample consisting of not more than 100 units shall be collected by taking single units at approximately equal intervals throughout the final production of the batch. If the person packaging the units into dispensing-size containers is not the manufacturer, the samples shall be collected by taking single units at approximately equal intervals during packaging. In no case shall more than 5,000 units be produced or packaged during a sampling interval. The minimum acceptable sample size shall be as specified in the appropriate monograph.

(*c*) When the manufacturing process is such that it is not feasible to collect the samples throughout the final production of the batch (e.g., if tablets undergo further processing, such as polishing or coating, after being compressed), the samples may be collected from bulk containers of the finished product, according to the following requirements:

(*1*) For batches exceeding 500,000 units: If the batch is in more than 100 containers, the sample is 1 unit from each container. If the batch is in 100 containers or less, the sample is 100 units, taken in approximately equal amounts from each container.

(*2*) For batches of 500,000 units or less: If the batch is in more than 100 containers, the sample is 1 unit from each container. If the batch is in 100 containers or less, the sample is at least 1 unit for every 5,000 units in the batch taken in approximately equal amounts from each container. The sample shall not be less than the minimum number of units specified in the appropriate monograph.

(iii) In the case of drugs packaged for repacking or for use in the manufacture of another drug, the sample must be representative of the batch. Such samples may be taken from a composite composed of portions taken from a

representative number of bulk containers, the composite consisting of no more than 10 times the amount required for conducting the required tests and assays. Such samples are not required if they have been previously submitted.

(iv) In the case of a sterile drug packaged in combination with containers of a sterile diluent, the sample shall be collected by taking 20 immediate containers of the diluent collected at regular intervals throughout each filling operation, except that if the diluent is sterilized after filling into containers, the representative sample shall consist of 20 immediate containers collected from each sterilizer load and each container shall be taken from a different part of each such sterilizer load. In the case of sterile drugs packaged in combination with sterile dispensers, the sample shall be collected by taking 20 dispensers from each sterilizer load, and each dispenser shall be taken from a different part of such sterilizer load.

(9) In the case of an initial request for certification, each ingredient used in making the batch other than ingredients required by specific regulations: 1 package of each containing approximately 5 grams. Results and dates of the latest tests and assays made by or for him on such ingredients shall precede or accompany the submission.

(10) The results and dates of tests and assays made by or for him on the nonantibiotic active ingredients in the batch.

(11) If such batch or any part thereof is to be packaged with a sterile diluent or sterile dispenser, such request shall also be accompanied by a statement that such diluent or dispenser is sterile and conforms to the requirements prescribed therefor by specific regulations.

(d) Each sample submitted pursuant to the regulations in this chapter shall be addressed to the Commissioner. Its package shall be clearly identified as to its contents and shall bear the name and post-office address of the person submitting it.

(e) In addition to the information and samples specifically required to be submitted to the Commissioner by the regulations in this chapter, the person who requests certification of a batch shall submit such further information and samples as the Commissioner may require for the purpose of investigations to determine whether or not such batch complies with the requirements of § 431.10 for the issuance of a certificate.

(f) Reference standards identical to working standards are available from: U.S.P. Reference Standards, 12601 Twinbrook Parkway, Rockville, Md. 20857, 301–881–0666.

[39 FR 18934, May 30, 1974, as amended at 41 FR 46852, Oct. 26, 1976; 43 FR 41195, 41197, Sept. 15, 1978; 45 FR 40111, June 30, 1980; 50 FR 7516, Feb. 22, 1985; 50 FR 8997, Mar. 6, 1985; 55 FR 11582, Mar. 29, 1990]

§ 431.5 Samples for sterility testing.

(a) *''Filling operation'' and ''sample'' defined.* (1) The term ''filling operation'' when used in connection with samples of a batch required for sterility testing refers to that period of time not longer than 24 consecutive hours during which a homogeneous quantity of drug is being filled continuously into market-size containers and during which no changes are made in the equipment used for filling. (Short rest periods for operators of the filling equipment and the time required to change operators between consecutive shifts are not considered as a break in continuity of the filling operation.) If more than one filling device is used during the filling operation, the samples shall include immediate containers filled by each device, and each such container shall be identified with a mark corresponding to that assigned to the filling device. If more than one filling operation is required to fill a batch, each container in the sample shall be identified with the number of the operation.

(2) For the purpose of sterility testing, the term ''sample'' means the total number of containers taken from each filling operation.

(b) *Packaging requirements for samples.* If a batch of a sterile antibiotic is packaged for repacking or for use as an ingredient in the manufacture of another drug, the sample required for sterility testing may be packaged in one container, in lieu of 20 containers, or in two containers in lieu of 40 containers, under the following conditions:

(1) The weight or volume of the sample is equivalent to the composite weight or volume required for a multiple container sample;

(2) The sample is a composite of samples taken from all parts of the batch; and

(3) The sterility test method prescribed for the drug by the regulations in this chapter is ''Bacterial membrane filter method'' described in § 436.20(e)(1) of this chapter.

§ 431.10 Certification.

(a) If it appears to the Commissioner, after such investigation as he considers necessary, that:

(1) The information (including results of tests and assays) and samples required by or pursuant to the regulations in this chapter have been submitted, and the request for certification contains no untrue statement of a material fact; and

(2) The batch complies with the regulations in this chapter and conforms to the applicable standards of identity, strength, quality, and purity prescribed by the regulations in this chapter;

the Commissioner shall certify that such batch is safe and efficacious for use, subject to such conditions on the effectiveness of certificates as are prescribed by § 431.11 and shall issue to the person who requested it a certificate to that effect.

(b) If the Commissioner determines, after such investigation as he considers to be necessary, that the information submitted pursuant to the regulations in this chapter, or the batch covered by such request, does not comply with the requirements set forth in paragraph (a) of this section for the issuance of a certificate, the Commissioner shall refuse to certify such batch and shall give notice thereof to the person who requested certification, stating his reasons for refusal.

(c) All statements, samples, and other information and materials submitted in connection with a request for certification shall be considered to be part of such request.

(d) Compliance of a drug with the standards of identity, strength, quality, and purity prescribed by regulations in this chapter shall be determined by the tests and methods of assay prescribed for such drug by regulations issued under this chapter.

(e) The regulations in this chapter, prescribing tests and methods of assay for antibiotic and antibiotic-containing drugs, shall not be construed as preventing the Commissioner from using any other test or method of assay in his investigations to determine whether or not:

(1) A request for certification contains any untrue statement of a material fact; or

(2) A certification has been obtained through fraud, or through misrepresentation or concealment of a material fact.

(f) Except as specifically provided by the regulations in this chapter, no provision of any regulation shall be construed as exempting any certifiable antibiotic drug from any applicable provision of the act or any regulation thereunder.

§ 431.11 Conditions on the effectiveness of certificates.

(a) A certificate shall not become effective:

(1) If it is obtained through fraud or through misrepresentation or concealment of a material fact;

(2) With respect to any package unless it complies with the packaging requirements, if any, prescribed by the regulations in this chapter which were in effect on the date of the certificate;

(3) With respect to any package unless its label and labeling bear all words, statements, and other information required by the regulations in this chapter; or

(4) With respect to any package of a certifiable antibiotic drug subject to the regulations in this chapter, when it is included in a packaged combination with another drug, unless such other drug complies with the requirements of the regulations in this chapter.

(b) A certificate shall cease to be effective:

(1) With respect to any immediate container after the expiration date, if any, prescribed by the regulations in this chapter;

(2) With respect to any immediate container when it or its seal (if the regulations in this chapter require it to be

sealed) is broken, or when its label or labeling is altered, multilated, destroyed, obliterated, or removed in whole or in part, or ceases to conform to any labeling requirement prescribed by the regulations in this chapter, except that:

(i) If the drug in such container is repacked or used as an ingredient in the manufacture of another drug, and certification of the batch thus made is requested, such certificate shall continue to be effective for a reasonable time to permit certification or destruction of such batch;

(ii) If the drug is in a container packaged for dispensing and is used in compounding a prescription issued by a practitioner licensed by law to administer such drug, such certificate shall continue to be effective for a reasonable time to permit the delivery of the drug compounded on such prescription; or

(iii) If its label or labeling is removed in whole or in part for the purpose of relabeling and supplemental certification of the relabeled drug is requested, as provided by §433.12 of this chapter.

(3) With respect to any immediate container of penicillin when it is included in the packaged combination penicillin with aluminum hydroxide gel or penicillin with a vasoconstrictor, or to any immediate container of bacitracin when it is included in the packaged combination bacitracin with a vasoconstrictor, except that when certification of the batch so included is requested, such certificate shall continue to be effective for a reasonable time to permit certification of such batch which is part of such combination;

(4) With respect to any package when the drug therein fails to meet the standards of identity, strength, quality, and purity which were in effect on the date of the certificate; except that those minor changes which occur before the expiration date and which are normal and unavoidable in good storage and distribution practice shall be disregarded.

(5) With respect to any package of a certifiable antibiotic drug subject to the regulations in this chapter, included in a packaged combination with another drug, when such other drug fails to meet the requirements of the regulations in this chapter; or

(6) With respect to any immediate container, if such regulations require its labeling to bear a caution against dispensing otherwise than on prescription, at the beginning of the act of dispensing or offering to dispense it otherwise than:

(i) By a practitioner licensed by law to administer such drug; or

(ii) On his prescription issued in his professional practice.

§431.12 Certification of antibiotic drugs after shipment in bulk containers.

(a) The Food and Drug Administration has received inquiries from certain interested manufacturers concerning their shipment of certified antibiotics, packaged in bulk containers, to hospitals and pharmacies for repacking or for use in the manufacture of another drug on the order or prescription of a physician. The regulations promulgated under section 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357) do not prohibit the shipment of certified bulk containers of antibiotics to such persons. However, under the provisions of §431.11(b)(2)(i), certification should be requested of each repacked batch and of each batch of another drug manufactured from such bulk drug, unless the repackaged drug or other drug has been made exempt from the certification requirements by regulation. The fact that the drug is to be repacked or manufactured on the order or prescription of a physician does not exempt it from the certification requirements of the act. Under the provisions of §431.11(b)(2)(ii), it is only when the drug used to compound a prescription is in a container packaged for dispensing that certification of the drug so compounded is not required.

(b) In the light of these provisions, unless the manufacturer and shipper of bulk containers of antibiotics has, with the consignee, an effective permit issued under §433.16 of this chapter, if the drug is to be repacked, or under §433.13 of this chapter if it is to be used in the manufacture of another drug, the shipper has the responsibility of seeing that certification is requested of

each repacked batch and of each batch of another drug manufactured from such drug.

§ 431.17 Request to provide for certification of an antibiotic drug.

A request under section 507 of the Federal Food, Drug, and Cosmetic Act to provide for certification of an antibiotic drug is required to comply with the procedures and meet the requirements applicable to the submission to the Food and Drug Administration and review by the agency of applications and abbreviated applications, and amendments and supplements to them, under part 314 of this chapter.

[50 FR 7516, Feb. 22, 1985]

§ 431.20 Disposition of outdated drugs.

When certification becomes invalid because the expiration date is passed, such articles should not be disposed of for drug use either through commercial or charitable channels unless the articles have been assayed to establish potency and recertified.

Subpart B—Administrative Procedures

§ 431.50 Forms for certification or exemption of antibiotic drugs.

The following forms which must be supplied in connection with certain certification or exemption procedures for antibiotic drugs may be obtained from the Product Surveillance Branch (HFD–333), Food and Drug Administration, Department of Health and Human Services, 5600 Fishers Lane, Rockville, MD 20857.

Form
1 Application for exemption for storage.
2 Application for exemption for processing.
3 Application for exemption for labeling.
4 Application for exemption for manufacturing use.
7 Request for check tests and assays or certification of a batch of _____(the blank to be filled in with the name of the antibiotic drug).
8 Application for exemption for repacking.
9 Request for supplemental certification of a batch of an antibiotic drug.

[39 FR 18934, May 30, 1974, as amended at 40 FR 28052, July 3, 1975; 41 FR 10886, Mar. 15, 1976; 50 FR 7516, Feb. 22, 1985; 50 FR 8997, Mar. 6, 1985; 55 FR 11582, Mar. 29, 1990]

§ 431.51 Suspension of certification service.

When the Commissioner finds that a person has:

(a) Obtained or attempted to obtain a certificate through fraud or through misrepresentation or concealment of a material fact; or

(b) Falsified the records required to be kept by § 431.61; or

(c) Failed to keep such records or to make them available, or to accord full opportunity to take an inventory of stocks on hand, or otherwise to check the correctness of such records as required by § 431.61; or

(d) Failed to establish a system for maintaining the records required by § 314.81 of this chapter or has repeatedly or deliberately failed to maintain such records or to make required reports in accordance with the provisions of that section, or has refused to permit access to, or copying, or verification of such records or reports; or

(e) Failed to conform to the requirements of good manufacturing practice prescribed by parts 210, 211, 225, 226 and 229 of this chapter;

the Commissioner will immediately suspend service to such person under the regulations in this chapter. Upon request a hearing will be granted to such person to show cause why such service should be resumed.

[39 FR 18934, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975; 55 FR 11582, Mar. 29, 1990]

§ 431.52 Hearings.

Any person who contests the suspension of certification service under § 431.51 shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

[41 FR 48267, Nov. 2, 1976, as amended at 42 FR 15675, Mar. 22, 1977]

§ 431.53 Fees.

(a) Fees for the services rendered under the regulations in this chapter shall be such as are necessary to provide, equip, and maintain an adequate certification service.

(b) The fee for such services with respect to each batch of a drug, certification of which is provided by the regulations in this chapter, shall be $114 for each batch submitted, plus the sum of the fees for all individual tests required for certification of each batch. The minimum tests for each batch shall be those prescribed in the section relating specifically to such drug.

(1) The fee schedule for specific tests required for antibiotic drug certification is as follows:

CHARGEABLE FEE PER TEST

Arquad content	$20
Benzylpenicilloyl content	32
Bleomycin	1,291
Butanol content	52
Candicidin potency (special turbidimetric)	85
Capreomycin 1 content	121
Color identity	8
Column chromatography	130
Column chromatographic isomer content	65
Copper content	22
Crystallinity	4
Cycloserine color assay	27
Daunorubicin potency (special turbidimetric)	19
Depressor substance test	40
Disc potency	52
Dissolution test	107
Doxycycline purity (paper chromatography)	130
Free chloride	54
Frozen antibiotic test panel	32
Gas chromatography	32
Gentamicin C	165
Heavy metals test	14
High pressure liquid chromatography (HPLC)	54
Infrared identity	19
Infrared quantitative	19
Iodochlorhydroxyquin content	22
Isoniazid content	22
Karl Fischer moisture	8
LD_{50} toxicity	185
Loss on drying	12
Lysine content	161
Melting range	8
Metal particles (ophthalmic ointments)	22
Microbiological assay, plate	50
Microbiological assay, turbidimetric	29
Microorganism count	68
Nonaqueous titrations (and compleximetric)	22
Paper chromatographic identity	43
Penicillenate and penamaldate content	30
Penicillin chemical assay	15
Penicillin contamination	39
Penicillin G content	32
pH	4
Polarographic assay	33
Potency (special plate)	91
Probenecid content	32
Procaine colorimetric	8
Pyrogens test: 3 rabbits	72
Pyrogens test: 8 rabbits	144
Quantitative thin layer chromatography	80
Residual streptomycin	8
Residue on ignition	26
Solubility identification	54
Specific rotation	22
Specific rotation (potency quantitative)	44
Specific surface area	22

CHARGEABLE FEE PER TEST—Continued

Sterility test	68
Sulfate content	8
Tablet disintegration	5
Thin layer chromatographic identity	43
Total Chlorine	54
Ultraviolet absorptivity	32
Ultraviolet identity	32
Ultraviolet potency	32
Vancomycin identity (bioautograph)	117
Zinc titration	11

(2) The fee for a supplemental request submitted pursuant to the provisions of § 433.12 of this chapter shall be $50.

(3) [Reserved]

(4) In the case of persons using the certification services and whose manufacturing facilities are not located in the United States or the Commonwealth of Puerto Rico, such persons shall be required to deposit each year sufficient funds to cover costs encountered when their facilities are inspected pursuant to the provisions of section 704 of the act.

(c) When the Commissioner considers it necessary to make investigations of a new product containing a certifiable antibiotic drug on which a request has been submitted in accordance with § 431.17, the fee for such service shall be the cost thereof. In such case the request shall be followed by an advance deposit in such amount as the Commissioner specifies, and thereafter such additional advance deposits shall be made as the Commissioner estimates may be necessary to prevent arrears in the payment of such fee.

(d) A person requiring continuing certification services may maintain an advance deposit of the estimated cost of such services for a two-month period. Such deposit shall be debited with fees for services rendered, but shall not be debited for any fee the amount of which is not definitely specified in the regulations in this chapter unless the depositor has previously requested the performance of the services to be covered by such fee. A monthly statement for each such advance deposit shall be rendered.

(e) The fees for the services rendered with respect to each batch certified under the regulations in this chapter shall accompany the request for certification, or the request for check tests and assays, unless such fee is covered by an advance deposit maintained in accordance with paragraph (d) of

this section. Also, if the Commissioner considers that investigations other than examination of such samples are necessary to determine whether or not such batch complies with the requirements of § 431.10 for the issuance of a certificate, the fee shall include the cost of such investigations.

(f) The unearned portion of any advance deposit shall be refunded to the depositor upon his application.

(g) Whenever in the judgment of the Commissioner the ratio between fees collected (which are based upon experience and the best estimate of costs and the best estimate of earnings) and the costs of providing the service during an elapsed period of time, in the light of all circumstances and contingencies, warrants a refund from the fund collected during such period, he shall make ratable refunds to those persons to whom the services were rendered and charged, except for those services described under § 433.12 of this chapter.

(h) All deposits and fees required by the regulations in this chapter, shall be paid by money order, bank draft, or certified check drawn to the order of the Food and Drug Administration, collectible at par at Washington, DC. All such deposits and fees shall be forwarded to the Food and Drug Administration, Department of Health and Human Services, Accounting Branch (HFA–120), 5600 Fishers Lane, Rockville, MD 20857, whereupon after making appropriate records thereof they will be transmitted to the Chief Disbursing Officer, Division of Disbursement, Treasurer of the United States, for deposit to the special account ''Salaries and Expenses, Certification, Inspection, and Other Services, Food and Drug Administration.''

[39 FR 18934, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975; 40 FR 28052, July 3, 1975; 41 FR 2384, Jan. 16, 1976; 41 FR 18291, May 3, 1976; 44 FR 67113, Nov. 23, 1979; 45 FR 16471, Mar. 14, 1980; 46 FR 16677, Mar. 13, 1981; 46 FR 60578, Dec. 11, 1981; 46 FR 61071, Dec. 15, 1981; 50 FR 19918, May 13, 1985; 55 FR 11582, Mar. 29, 1990]

Subpart C—Records and Reports

§ 431.61 Records of distribution.

(a) The person who requested certification shall keep complete records showing each shipment and other delivery (including exports) of each certified batch or part thereof by such person or by any person subject to his control. Such records shall show the date and quantity of each such shipment or delivery and the name and post-office address of the person to whom such shipment or delivery was made, and shall be kept for not less than 3 years after such date.

(b) Upon the request of any officer or employee of the Food and Drug Administration, or of any other officer or employee of the United States acting on behalf of the Secretary, the person to whom a certificate is issued shall at all reasonable hours make such records available to any such officer or employee and shall accord to him full opportunity to make inventory of stocks of such batch on hand and otherwise to check the correctness of such records.

§ 431.62 Records retention.

At the option of the person having control of records required to be kept by any regulation in this part 431, photostatic or other permanent reproductions may be substituted for such records after the first 2 years of the holding period.

Subpart D—Confidentiality of Information

§ 431.70 Confidentiality of data and information in an investigational new drug notice for an antibiotic drug.

(a) The existence of an IND notice for an antibiotic drug will not be disclosed by the Food and Drug Administration unless it has previously been publicly disclosed or acknowledged.

(b) The availability for public disclosure of all data and information in an IND file for an antibiotic drug shall be handled in accordance with the provisions established in § 314.430 of this chapter.

(c) Notwithstanding the provisions of § 314.430 of this chapter, the Food and Drug Administration shall disclose upon request to an individual on whom an investigational antibiotic has been used a copy of any adverse reaction report relating to such use.

[39 FR 44655, Dec. 24, 1974, as amended at 50 FR 7517, Feb. 22, 1985]

PART 432—PACKAGING AND LABELING OF ANTIBIOTIC DRUGS

Sec.
432.1 Packaging requirements.
432.5 Labeling requirements.
432.9 Labeling of antibiotic drugs intended for export.
432.20 Declaration of potency.

AUTHORITY: 21 U.S.C. 321, 331, 352, 353, 357, 371, 381.

CROSS REFERENCE: For other regulations in this chapter concerning antibiotic drugs exempted from certain labeling requirements, see also § 201.150 of this chapter.

§ 432.1 Packaging requirements.

Each antibiotic drug subject to certification under section 507 or 512(n) of the act shall be packaged in immediate containers which shall be of such composition as not to cause any change in the strength, quality, or purity of the contents beyond any limits therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. The immediate containers shall be tight containers as defined by the U.S.P., except that if the antibiotic drug is dispensed as an ointment or cream, the immediate containers shall be well-closed containers as defined by the U.S.P. If the antibiotic drug is packaged for dispensing, it may be packaged in combination with a container of a suitable and harmless diluent approved by the Commissioner.

(a) If it is a sterile preparation, the containers shall be sterile at the time of filling and closing and shall be so sealed that the contents cannot be used without destroying the seal.

(b) If it is intended for parenteral use and the container is glass, it shall be transparent and colorless or light-resistant as defined by the U.S.P. The containers are closed either by fusion or by application of suitable closures, in such manner as to prevent contamination or loss of content. Multiple-dose containers are closed by a substance through which a hypodermic needle may be introduced and withdrawn without removing the closure or destroying its effectiveness. Each container shall be filled with a quantity of a volume in excess of that designated, which excess shall be sufficient to permit the withdrawal and administration of the labeled quantity or volume, whether administered in single or multiple doses.

(c) If it is dispensed as a tablet, capsule, troche, pellet, or suppository, it may be enclosed in a foil or plastic film and such enclosure is a tight container as defined by the U.S.P., except for the provision that it shall be capable of tight reclosure. The immediate container may contain a dessicant separated from the drug by a plug of cotton or other like material.

(d) If it is dispensed as an ointment or cream, it shall be in collapsible tubes that shall in no case be larger than the 2-ounce size, except:

(1) If it is labeled for institutional use, it may be packaged in immediate containers larger than the 2-ounce size and it may be packaged in immediate containers of glass or plastic; or

(2) If it is an ointment represented for ophthalmic use, it shall be in collapsible tubes which shall not be larger than the ⅛-ounce size.

(e) If it is intended for ophthalmic use, the closure shall be one through which a hypodermic needle cannot be introduced.

[39 FR 18938, May 30, 1974, as amended at 42 FR 44225, Sept. 2, 1977; 44 FR 10377, Feb. 20, 1979]

§ 432.5 Labeling requirements.

(a) If an antibiotic drug is packaged for dispensing:

(1) It shall be labeled in accordance with the requirements prescribed by § 201.100 of this chapter, issued under section 502(f) of the act, unless the regulations pertaining to such drug specifically exempt it from such requirements.

(2) Its labeling shall bear any additional information required for the drug by specific regulations.

(3) Each package shall bear on its outside wrapper or container and the immediate container an expiration date prescribed for the drug by specific regulations; except that in lieu of the expiration date prescribed by specific regulations, a date may be used that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the

batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the specific regulation does not stipulate an expiration period, it shall be as prescribed by this section. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker. If an expiration date is used that is longer than the minimum date provided for the drug by specific regulations, it may be used only if the manufacturer has submitted information to the Commissioner adequate to prove that the drug is stable for such time.

(b) If it is packaged solely for manufacturing use or for repacking, each package shall bear on its outside wrapper or container and the immediate container, the following:

(1) The number of units or micrograms of activity per milligram or per gram, and the number of grams or kilograms in the immediate container.

(2) The batch mark.

(3) The statement ''Caution: Federal law prohibits dispensing without prescription.''

(4) The statement ''For manufacturing use,'' ''For repacking,'' or ''For manufacturing use or repacking,'' and, if it is not sterile, the statement ''nonsterile.''

(5) The required expiration date.

(c) The expiration date prescribed for a drug by the regulations in this chapter may be omitted from the label of the immediate container if such container contains a single dose and it is packaged in an individual wrapper or container that bears the date prescribed.

[39 FR 18938, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 432.9 Labeling of antibiotic drugs intended for export.

(a) Antibiotic drugs subject to certification under section 507 of the act and intended for export will be certified notwithstanding failure to meet the labeling requirements of the applicable sections if the labeling used for such drugs meets the following conditions:

(1) It has been approved before use by the Government authorities of the country to which the drugs are intended for export; and

(2) Such labeling represents that such drugs are for use only in those conditions for which they are certified for domestic distribution.

(b) The legend ''Caution: Federal law prohibits dispensing without prescription'' might be inappropriate on antibiotic drugs exported from the United States, since their sale may or may not be so restricted under the laws of the country of destination. The Food and Drug Administration would not object to a slight modification of the wording to read, ''Caution: Federal (U.S.A.) law prohibits dispensing without prescription,'' by a manufacturer who wishes to market a drug under the same label both in domestic and foreign commerce.

[39 FR 18938, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 432.20 Declaration of potency.

Wherever the potency of an antibiotic drug included in the regulations in this chapter is expressed in terms of weight, such potency shall be equivalent to that contained in the same weight of the master standard of the drug.

[39 FR 18938, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

PART 433—EXEMPTIONS FROM ANTIBIOTIC CERTIFICATION AND LABELING REQUIREMENTS

Subpart A—General Provisions

Sec.

433.1 Exemption of antibiotic drugs for human use from batch certification requirements.
433.2 Conditions on the effectiveness of exemptions of antibiotic drugs for human use from batch certification requirements.
433.3 Assay requirements for antibiotic drugs exempted from certification.

Subpart B—Exemptions for Which an Application or Notice Is Required

433.12 Exemption for labeling.
433.13 Exemption for manufacturing use.
433.14 Exemption for storage.
433.15 Exemption for processing.
433.16 Exemption for repacking.
433.17 Exemption for investigational use.

Subpart C—Specific Use Exemptions

433.20 Antibiotic drugs for isolation and differentiation of microorganisms in clinical use.
433.21 Antibiotics for diagnostic use.
433.22 Biologic drugs that contain antibiotics as a preservative.
433.23 Microbiological culture media containing antibiotics.
433.24 Exemption of antibiotic drugs for use in teaching, law enforcement, research and analysis.
433.25 [Reserved]
433.26 Neomycin sulfate ointment intended for hypersensitivity testing.

Subpart D—Records and Reports

433.30 Records retention.

AUTHORITY: 21 U.S.C. 352, 355, 357.

SOURCE: 39 FR 18939, May 30, 1974, unless otherwise noted.

Subpart A—General Provisions

§433.1 Exemption of antibiotic drugs for human use from batch certification requirements.

(a) Antibiotic drugs for human use are exempt from the batch certification requirements of part 431 of this chapter if the conditions of paragraph (b) of this section are met; or, in the case of over-the-counter antibiotic drugs subject to an over-the-counter drug monograph in this chapter, if the conditions of paragraph (c) of this section are met.

(b) The conditions are as follows:

(1) The antibiotic drug is approved for marketing under an appropriate antibiotic application or abbreviated antibiotic application or is the subject of review under the Drug Efficacy Study Implementation Program.

(2) The antibiotic drug is packaged and labeled for dispensing in accordance with the applicable regulation (monograph) in this chapter except where other labeling has been approved in an applicable antibiotic application or abbreviated antibiotic application.

(3) The bulk antibiotic drug used in preparing the antibiotic drug product meets the standards of identity, strength, quality, and purity specified in the applicable regulation (monograph) in this chapter except where other standards have been approved in an applicable antibiotic application or abbreviated antibiotic application.

(4) The antibiotic drug product meets the standards of identity, strength, quality, and purity specified in the applicable regulation (monograph) in this chapter except where other standards have been approved in an applicable antibiotic application or abbreviated antibiotic application.

(c) The over-the-counter antibiotic drug product for human use is required to meet the general conditions established in §330.1 of this chapter, and the conditions specified in an applicable over-the-counter drug monograph in this chapter.

(d) In accordance with the provisions of section 507(e) of the act, an antibiotic-containing drug for human use exempt from the requirements for batch certification under paragraph (b) of this section is subject following its approval to section 505 of the act and applicable regulations for new drugs, generally parts 310 through 314 of this chapter. For each antibiotic drug subject to an exemption under paragraph (b) of this section:

(1) An approved antibiotic application is regarded to be an approved application under §314.50 of this chapter.

(2) An approved abbreviated antibiotic application is regarded to be an approved abbreviated application under §314.94 of this chapter.

(e) Nothing in this section prevents a manufacturer from applying for batch certification of an antibiotic drug for human use subject to an exemption under this section as provided in section 507(c) of this act.

(f) All exemptions from batch certification requirements for antibiotic drugs for human use under this section

are subject to the conditions of effectiveness under § 433.2.

(Approved by the Office of Management and Budget under control number 0910–0001)

[51 FR 25524, July 15, 1986; 51 FR 30478, Aug. 27, 1986, as amended at 57 FR 18001, Apr. 28, 1992]

§ 433.2 Conditions on the effectiveness of exemptions of antibiotic drugs for human use from batch certification requirements.

(a) If at any time an exemption from batch certification requirements for an antibiotic drug for human use has been granted, the Commissioner finds on the basis of new information before the agency with respect to such exempted drug, evaluated together with the evidence available to the agency when such exemption was granted, that certification of each batch is necessary to ensure its safety and efficacy of use, the Commissioner shall act immediately to revoke all exemptions from batch certification requirements granted for such drug.

(b) If the Commissioner finds that the person granted an exemption from batch certification requirements for an antibiotic drug for human use has failed either to comply with the requirements of section 505 of the act and the regulations promulgated thereunder or to meet the general conditions established in § 330.1 of this chapter and the conditions specified in an applicable over-the-counter drug monograph in this chapter; or if the Commissioner finds that the requirements of § 433.1 have not been met; or if the Commissioner finds that the petition for exemption from batch certification contains any false statements of fact, the Commissioner may revoke the exemption from batch certification requirements immediately and require batch certification of the drug until such person shows adequate cause why the exemption from batch certification requirements should be reinstated.

(c) If the Commissioner repeals or suspends an exemption from batch certification requirements for an antibiotic drug for human use, a notice to that effect and the reasons therefor will be published in the FEDERAL REGISTER.

(d) Any person who contests the revocation or suspension or denial of reinstatement of an exemption from batch certification requirements for an antibiotic drug for human use shall have an opportunity for a regulatory hearing before the Food and Drug Administration under part 16 of this chapter.

[47 FR 39159, Sept. 7, 1982, as amended at 51 FR 25524, July 15, 1986]

§ 433.3 Assay requirements for antibiotic drugs exempted from certification.

(a) Certain antibiotic drugs are exempted by regulations in this chapter from the certification requirements of sections 507 and 512 of the act if such drugs comply with standards prescribed by such regulations and on condition that the label of each package bears an expiration date which is determined from the date during which the batch was last assayed and released by the manufacturer.

(b) It is the position of the Food and Drug Administration that if each batch of such exempted drugs is not tested by the manufacturer or his agent to determine whether it complies with the standards of identity, strength, quality, and purity prescribed for it, the batch is not exempt from certification and it may be deemed to be misbranded under section 502(l) of the act or be adulterated under section 501(a)(5) of the act when in interstate commerce.

Subpart B—Exemptions for Which an Application or Notice Is Required

§ 433.12 Exemption for labeling.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a certifiable antibiotic drug which is to be labeled at an establishment located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in such establishment, from the requirement of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each shipping container bears the batch mark of the drug, the number of units per package

and the expiration date, and if the person who introduced such shipment or delivery into interstate commerce holds a permit (Antibiotic Form 3) from the Commissioner authorizing shipment for labeling in such establishment.

(b)(1) An application for such a permit shall be in a form specified by the Commissioner and shall give the name and location of the establishment in which such labeling is to be done.

(2) In case the applicant is the operator of such establishment, the application shall include a written agreement signed by him that he will request certification of each batch from which any shipment or delivery is made to such establishment unless it is exempt under section 801(d) of the act or § 433.17; that he will not remove any of such antibiotic drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt, or if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records showing the date, quantity, and batch mark of each such shipment and delivery and the disposition thereof; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition; and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

(3) In case the applicant is not the operator of such establishment such application shall include or be accompanied by:

(i) A written agreement signed by the applicant that he will request certification of each batch from which any shipment or delivery is made to such establishment unless it is exempt under section 801(d) of the act or § 433.17; that he will keep complete records showing the date, quantity, and batch mark of each such shipment and delivery; and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery; and

(ii) A written agreement signed by the operator of such establishment that he will submit a request, supplemental to that of the applicant, for the certification of each batch or portion thereof comprised in any such shipment or delivery received by him unless it is exempt under section 801(d) of the act or § 433.17; that he will specify in his request the number of packages of each size in such shipment or delivery, the date of delivery, the batch mark thereof, and the batch mark he will use therefor; that the batch marks to be used (if different from those of the applicant) will be only those of which the key is specified in this agreement; that the expiration date used for the batch will be only that assigned to the manufacturer by certification; that the labeling to be used for such packages will be only that of which specimens are attached to this agreement (including specimens of all brochures and other printed matter, except readily available medical publications, referred to in such labeling); that when any change is made in such key or labeling he will promptly submit to the Commissioner a full statement of such change or, in the case of changed labeling, specimens showing all such changes; that he will not remove any of such antibiotic drug from such establishment unless it complies with section 502(l) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records of the disposition of each such shipment and delivery; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition; and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

(4) When the Commissioner finds that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit.

(5) Any person who contests the denial or revocation of a permit shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after labeling, from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who inintroduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery of any part thereof, before or after labeling from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.17 or, if certification is refused, unless such shipment or delivery, within a reasonable time, is destroyed or returned to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

[39 FR 18939, May 30, 1974, as amended at 41 FR 48267, Nov. 2, 1976; 42 FR 15675, Mar. 22, 1977]

§ 433.13 Exemption for manufacturing use.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter that is packed in containers of not less than 10,000,000 units of penicillin or 10 grams each of one of the other antibiotic drugs shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in the establishment where it is so used, from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act, if it conforms to the standards prescribed therefor by the section of the regulations in this chapter which is specifically applicable to such other antibiotic drug, if the label of each container bears the batch mark of the drug, the number of units or grams per package, and the date on which the latest assay of the drug was completed, and if the person who introduced each shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for manufacturing use in such establishment.

(b) An application for such a permit shall be in a form specified by the Commissioner, shall give the name and location of the establishment in which such drug is to be used and shall be accompanied by;

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery;

(2) A written statement signed by the operator of such establishment showing that he has adequate facilities for the manufacture of such other drug; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof and showing the quantity and batch mark of each batch

of such other drug manufactured by him and the disposition thereof; that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after its manufacture is completed that he will request certification of each batch thereof unless it is exempt under section 801 (d) of the act or § 433.12, § 433.14, § 433.16, or § 433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or is returned to him for labeling.

When the Commissioner finds that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated, he may revoke such permit. Any person who contests the denial or revocation of a permit shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such establishment, prior to its use in the manufacture of another drug, unless it is exempt under section 801(d) of the act.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such establishment, prior to its use in the manufacture of another drug, unless it is exempt under section 801(d) of the act.

[39 FR 18939, May 30, 1974, as amended at 41 FR 48267, Nov. 2, 1976; 42 FR 15675, Mar. 22, 1977]

§ 433.14 Exemption for storage.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a drug which is to be stored at a warehouse located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in such warehouse, from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each shipping container bears the batch mark of the drug, and if the person who introduced such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for storage in such warehouse.

(b) An application for such a permit shall be in a form specified by the Commissioner, and shall give the name and location of the warehouse in which such drug is to be stored. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or § 433.12, § 433.13, or § 433.16, that he will not remove any of such drug from such warehouse unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or, if certification is refused unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured; that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such warehouse, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery; and

(2) A written statement signed by the operator of such warehouse showing

that he has adequate facilities for such storage; such statement shall contain an agreement that he will hold each shipment or other delivery of such drug intact, under such conditions as will not cause failure of the drug to comply with the requirements for certification, that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records.

If the applicant keeps complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug from such warehouse and the name and post-office address of the person to whom such shipment or delivery was made, the agreement to keep records of such disposals, to make such records available, and to afford opportunity for checking their correctness may be included in the applicant's agreement and omitted from that of the operator. When the Commissioner finds that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit. Any person who contests the denial or revocation of a permit shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such warehouse, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such warehouse unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.12, § 433.13, or § 433.16, or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such warehouse, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof from such warehouse unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.12, § 433.13, or § 433.16, or, if certification is refused, unless such shipment or delivery within a reasonable time, is destroyed, or returned to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

[39 FR 18939, May 30, 1974, as amended at 41 FR 48267, Nov. 2, 1976; 42 FR 15675, Mar. 22, 1977]

§ 433.15 Exemption for processing.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter in concentrated aqueous solution which is to be processed at an establishment located elsewhere than at the place of manufacture shall be exempt during the time of introduction into and movement in interstate commerce and the time of holding in such establishment from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act, if the person who introduced such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for processing in such establishment, and each package of such solution bears the batch mark of the drug.

(b) An application for such a permit shall be in a form specified by the Commissioner and shall give the name and location of the establishment in which

such processing is to be done. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, potency, and batch mark of each shipment and other delivery of any such solution to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such shipment or delivery;

(2) A written agreement signed by the operator of such establishment showing that he has adequate facilities for such processing; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after the processing is completed that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or §433.12, §433.13, §433.14, §433.16, or §433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt.

When the Commissioner finds that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit. Any person who contests the denial or revocation of a permit shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after processing, from such establishment unless the batch made from such shipment or delivery complies with section 502(l) of the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or §433.12, §433.13, §433.14, §433.16, or §433.17, or, if certification is refused, unless such shipment or delivery is reprocessed and certified or destroyed within a reasonable time.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after processing, from such establishment unless the batch made from such shipment or delivery complies with section 502(l) of the act or is exempt under section 801(d) of the act or the certification requirements of section 512(n) of the act or §433.12, §433.13, §433.14, §433.16, or §433.17, or, if certification has been refused, unless such shipment or delivery is reprocessed and certified or destroyed within a reasonable time.

[39 FR 18939, May 30, 1974, as amended at 41 FR 48267, Nov. 2, 1976; 42 FR 15675, Mar. 22, 1977]

§433.16 Exemption for repacking.

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of a drug which is to be repacked at an establishment located elsewhere than at the place of manufacture shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding such establishment from the requirements of section 502(l) of the act or the certification requirements of section 512(n) of the act if the labeling of each container bears the batch mark of the drug and the number of units per package, and if the person

who introduces such shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for repacking in such establishment.

(b) An application for such a permit shall be in a form specified by the Commissioner, and shall give the name and location of the establishment in which such repacking is to be done. Such application shall be accompanied by:

(1) A written agreement signed by the applicant that he will keep complete records showing the date, quantity, and batch mark of each shipment and other delivery of any such drug to such establishment, and that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of each shipment or delivery;

(2) A written statement signed by the operator of such establishment showing that he has adequate facilities for such repacking; such statement shall contain an agreement that he will keep complete records showing the date of receipt by him and the quantity and batch mark of each such shipment and delivery and the disposition thereof, that he will make such records available to any officer or employee of the Food and Drug Administration at any reasonable hour within 3 years after the date of such disposition, and that he will accord full opportunity to such officer or employee to make inventories of stocks on hand and otherwise check the correctness of such records; and

(3) A written agreement signed by the person who will own the drug after the repacking is completed that he will request certification of each batch thereof unless it is exempt under section 801(d) of the act or § 433.12, § 433.13, § 433.14, or § 433.17, and that he will not remove any of such drug from such establishment unless it complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is so exempt or is returned to him for labeling or, if certification is refused, unless it is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

When the Commissioner finds that such application contains any untrue statement of a material fact or that any provision of any such agreement has been violated he may revoke such permit. Any person who contests the denial or revocation of a permit shall have an opportunity for a regulatory hearing before the Food and Drug Administration pursuant to part 16 of this chapter.

(c) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is the operator of such establishment, shall become void at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after repacking, from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.12, § 433.13, § 433.14, or § 433.17, or is returned to such person for labeling or, if certification is refused, unless such shipment or delivery is returned within a reasonable time to permit reprocessing and certification, destruction, or such exemption at the establishment where it was manufactured.

(d) An exemption of a shipment or other delivery under paragraph (a) of this section, in case the person who introduced such shipment or delivery into interstate commerce is not the operator of such establishment, shall expire at the beginning of the act of removing or offering to remove such shipment or delivery or any part thereof, before or after repacking, from such establishment unless such batch complies with section 502(l) of the act or the certification requirements of section 512(n) of the act or is exempt under section 801(d) of the act or § 433.12, § 433.13, § 433.14, or § 433.17, or is returned to such person for labeling or, if certification is refused, unless such shipment or delivery within a reasonable time, is destroyed or returned to permit reprocessing and certification, destruction, or such exemption at the

establishment where it was manufactured.

[39 FR 18939, May 30, 1974, as amended at 41 FR 48268, Nov. 2, 1976; 42 FR 15675, Mar. 22, 1977]

§ 433.17 Exemption for investigational use.

A shipment or other delivery of an antibiotic drug shall be exempt from section 502(l) of the act or the certification requirements of section 512(n) of the act if all the procedures outlined in part 312 or § 511.1 of this chapter are complied with. For the purposes of this section, the references in part 312 or § 511.1 of this chapter to ''new drug'' and ''approved new animal drug application'' shall be deemed to read ''antibiotic drug'' and ''approval for certification or exemption from certification'' respectively.

[39 FR 18939, May 30, 1974, as amended at 40 FR 13497, May 27, 1975; 55 FR 11582, Mar. 29, 1990]

Subpart C—Specific Use Exemptions

§ 433.20 Antibiotic drugs for isolation and differentiation of microorganisms in clinical use.

Antibiotic drugs subject to section 507 of the act shall be exempt from section 502(l) if such drugs are:

(a) Paper discs impregnated with antibiotics in the amounts listed in the following table:

Antibiotic	Content per disc
Bacitracin	0.04 unit.
Nystatin	100 units.

(b) Packaged in a container bearing on its label or labeling the following:

(1) On the outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The potency of each disc in the batch.

(iii) The expiration date as prescribed under § 432.5(a)(3) of this chapter.

(iv) The statement: Not for Susceptibility Testing.

(2) On the labeling within or attached to the package: Adequate directions for use.

§ 433.21 Antibiotics for diagnostic use.

Antibiotics packaged for the withdrawal of individually weighed portions and intended for use solely in laboratory procedures in connection with the diagnosis or treatment of disease and conspicuously so labeled shall be exempt from the certification requirements of section 502(l) and 507 of the act and the certification requirements of section 512(n) of the act if they comply with all the following conditions:

(a) The potency, moisture content, and identity comply with the standards prescribed for the antibiotic by the specific regulations issued in this chapter.

(b) It is packaged in immediate containers that are tight containers as defined by the U.S.P. Each such container shall contain not more than 1 gram.

(c) Each package bears on the label or labeling of its outside wrapper or container and the immediate container the following:

(1) The statements ''For the withdrawal of individual portions of antibiotic. Each portion must be weighed before use. Diagnostic reagent. For professional use only.''

(2) The number of milligrams or grams contained in each immediate container and the potency per milligram.

(3) The batch mark.

(4) The statement ''Expiration date _____'', the blank being filled in with the date that does not exceed the expiration date authorized for the antibiotic by this chapter.

(d) The circular or other labeling within or attached to the package bears directions adequate for the use of such drug.

CROSS REFERENCES: For tests and methods of assay and certification of antibiotics susceptibility discs for laboratory diagnosis of disease, see §§ 460.1 and 460.6 of this chapter.

§ 433.22 Biologic drugs that contain antibiotics as a preservative.

Biological drugs that contain any certifiable antibiotic drug subject to the regulations in this chapter, and the purpose of the antibiotic is for use only as a preservative and the biological drug is conspicuously so labeled, shall be exempt from the requirements of sections 502(l) and 507 of the act and

the certification requirements of section 512(n) of the act, if such drugs are licensed under the Public Health Service Act of July 1, 1944 (58 Stat. 682; 42 U.S.C. 201 et seq.) or under the Virus-Serum-Toxin Act of March 4, 1913 (37 Stat. 832; 21 U.S.C. 151 et seq.).

§ 433.23 Microbiological culture media containing antibiotics.

Microbiological culture media that contain any certifiable antibiotic drug subject to the regulations in this chapter shall be exempt from the requirements of sections 502(l) and 507 of the act and the certification requirements of section 512(n) of the act if:

(a) They are intended for use in tissue culture and the antibiotic drug is added solely for use as an aid in the prevention of microbial contamination; or

(b) They are intended for use in the isolation of selected organisms from mixed cultures and the antibiotic drug is added solely for use as an aid in such isolation; and

(c) The certifiable antibiotic drug used in such culture media complies with the applicable standards of identity, strength, quality, and purity prescribed therefor.

§ 433.24 Exemption of antibiotic drugs for use in teaching, law enforcement, research, and analysis.

Antibiotic drugs subject to section 507 or 512(n) of the act shall be exempt from the requirements of section 502(l) and from the certification requirements of section 512(n) of the act if shipped or sold to, or in the possession of, persons regularly and lawfully engaged in instruction in pharmacy, chemistry, or medicine not involving clinical use; or in law enforcement; or in research not involving clinical use; or in chemical analysis or physical testing, provided they are to be used only for such instruction, law enforcement, research, analysis, or testing, and provided further that their labels bear the statement ''Not for drug use.''

§ 433.25 [Reserved]

§ 433.26 Neomycin sulfate ointment intended for hypersensitivity testing.

Neomycin sulfate ointment subject to sections 502(l) and 507 of the act and packaged for use as an allergen for skin patch testing of hypersensitivity shall be exempt from the certification requirements of section 502(l) and 507 of the act if it complies with all the following conditions:

(a) It contains neomycin sulfate equivalent to 200 milligrams of neomycin per gram in petrolatum.

(b) The neomycin sulfate used in preparing the neomycin sulfate ointment conforms to the standards prescribed by § 444.42(a)(1) of this chapter except § 444.42(a)(1)(ii).

(c) The shipment of neomycin sulfate is made as a result of a specific request made to the manufacturer or distributor by a practitioner licensed by law to administer such drug, and the use of neomycin sulfate ointment for patch testing is not promoted by the manufacturer or distributor.

(d) Each package shall bear on its outside wrapper or container and on the immediate container, in addition to other labeling information required by the act and regulations, the following statements in lieu of adequate directions for use:

(1) The statement, ''Caution: Federal law prohibits dispensing without prescription''.

(2) The statement, ''For use only in patch testing''.

(3) The potency of the ointment.

(4) The expiration date as prescribed by § 432.5(a)(3) of this chapter.

(e) The quantity shipped is limited to an amount reasonable for the purpose of patch testing in the normal course of the practice of medicine and is used solely for such patch testing.

(f) The manufacturer or distributor maintains records of all shipments for this purpose for a period of 2 years after shipment and will make them available to the Food and Drug Administration upon request.

[43 FR 11151, Mar. 17, 1978]

Subpart D—Records and Reports

§ 433.30 Records retention.

At the option of the person having control of records required to be kept by any regulation in this part 433, photostatic or other permannnt reproductions may be substituted for such

records after the first 2 years of the holding period.

PART 436—TESTS AND METHODS OF ASSAY OF ANTIBIOTIC AND ANTIBIOTIC-CONTAINING DRUGS

Sec.

Subpart A—Definitions; Interpretations; Requirements

436.1 Sterility requirements of items packaged with sterile antibiotic drugs.
436.2 Alternative assay methods.

Subpart B—Sterility Test Methods

436.20 Sterility test methods and procedures.

Subpart C—Biological Test Methods

436.31 Equipment and diluents for use in biological testing.
436.32 Pyrogen test.
436.35 Depressor substances test.

Subpart D—Microbiological Assay Methods

436.100 Laboratory equipment.
436.101 Solutions.
436.102 Culture media.
436.103 Test organisms.
436.104 Penicillin activity.
436.105 Microbiological agar diffusion assay.
436.106 Microbiological turbidimetric assay.

Subpart E—General Chemical Tests for Antibiotics

436.200 Loss on drying.
436.201 Moisture determination.
436.202 pH.
436.203 Crystallinity.
436.204 Iodometric assay.
436.205 Hydroxylamine colorimetric assay.
436.206 Test for metal particles in ophthalmic ointments.
436.207 Residue on ignition.
436.208 Heavy metals determination.
436.209 Melting range or temperature.
436.210 Specific rotation.
436.211 Identity tests by infrared spectrophotometry.
436.212 Disintegration test.
436.213 Nonaqueous titrations.
436.214 Heat stability.
436.215 Dissolution test.
436.216 High–performance liquid chromatographic assay.
436.217 Film-coat rupture test.

Subpart F—Chemical Tests for Specific Antibiotics

436.300 Polarimetric assay of carbenicillin indanyl sodium.
436.301 Thin layer chromatography identity test for carbenicillin indanyl.
436.302 Clindamycin vapor phase chromatography.
436.303 Clindamycin content of clindamycin palmitate hydrochloride by vapor phase chromatography.
436.304 Clindamycin phosphate vapor phase chromatography.
436.305 Thin layer chromatographic identity test for hetacillin.
436.306 Lincomycin gas liquid chromatography.
436.307 Spectinomycin vapor phase chromatography.
436.308 Paper chromatography identity test for tetracyclines.
436.309 Anhydrotetracyclines and 4-epianhydrotetracycline.
436.310 Thin layer chromatography identity test for mitomycin.
436.311 Thin layer chromatography identity test for amoxicillin.
436.312 Atomic absorption method for determining the zinc content of zinc bacitracin.
436.316 Determination of penicillin G content.
436.317 Solubility characteristic test for griseofulvin (ultramicrosize) tablets.
436.318 Continuous flow thin layer chromatography identity test.
436.319 Thin layer chromatography identity test for bacitracin and bacitracin zinc.
436.320 Ferric chloride colorimetric assay.
436.321 Griseofulvin gas liquid chromatography.
436.322 High–pressure liquid chromatographic assay for anthracycline antibiotics.
436.323 Continuous flow thin layer chromatography identity test for cefamandole nafate.
436.324 Polarographic analysis of cefamandole.
436.325 High pressure liquid chromatography assay for vidarabine.
436.326 Thin layer chromatographic identity test for cefoxitin sodium.
436.327 Thin layer chromatographic identity test for cyclacillin.
436.328 High pressure liquid chromatographic assay for sulfisoxazole acetyl content.
436.329 High-pressure liquid chromatographic assay for meclocycline.
436.330 Thin layer chromatographic identity test for bacampicillin.
436.331 High-pressure liquid chromatographic assay for dactinomycin.
436.332 High-pressure liquid

chromatographic assay for moxalactam.
436.333 Thin layer chromatographic identity test for moxalactam.
436.334 High-pressure liquid chromatographic assay for piperacillin.
436.335 High-pressure liquid chromatographic assay for chloramphenicol palmitate.
436.336 Thin layer chromatographic identity test for azlocillin.
436.337 High-pressure liquid chromatographic assay for cephradine.
436.338 High-pressure liquid chromatographic assay for cefoperazone.
436.339 High-pressure liquid chromatographic assay for bleomycin fractions.
436.340 High-pressure liquid chromatographic assay for tetracycline hydrochloride content and 4–epitetracycline hydrochloride content.
436.341 High-pressure liquid chromatographic assay for plicamycin.
436.342 High-pressure liquid chromatographic assay for cefazolin.
436.343 High-pressure liquid chromatographic assay for cefuroxime.
436.344 Thin layer chromatographic identity test for cefuroxime.
436.345 High-pressure liquid chromatographic assay for ceftizoxime.
436.346 High-pressure liquid chromatographic assay for cyclosporine.
436.347 High-pressure liquid chromatographic assay for cefoxitin.
436.348 High-pressure liquid chromatographic assay for ceforanide.
436.349 High-pressure liquid chromatographic assay for L-lysine in ceforanide for injection.
436.350 High-performance liquid chromatographic assay for cefonicid.
436.351 High-performance liquid chromatographic assay for amoxicillin and clavulanic acid.
436.352 High-performance liquid chromatographic assay for determining clavam-2-carboxylate content in clavulanate potassium.
436.353 High-performance liquid chromatographic assay for amdinocillin.
436.354 High-performance liquid chromatographic assay for ceftriaxone.
436.355 High–performance liquid chromatographic assay for ticarcillin–clavulanic acid.
436.356 High–performance liquid chromatographic assay for ceftazidime.
436.357 Atomic absorption test for sodium carbonate content.
436.358 High–performance liquid chromatographic assay for pyridine.
436.360 Gel permeation chromatographic assay for high molecular weight polymer.
436.361 High–performance liquid chromatographic assay for aztreonam.
436.362 Thin-layer chromatographic test for free erythromycin content in erythromycin estolate bulk.
436.363 High-performance liquid chromatographic assay for cefmenoxime.
436.364 Atomic absorption test for sodium carbonate content of cefmenoxime hydrochloride for injection.
436.365 Thin layer chromatographic identity test for rifampin.
436.366 High-performance liquid chromatography assay for determining chromatographic purity of vancomycin.
436.367 Thin-layer chromatographic identity test for cephalexin hydrochloride.
436.368 Thin layer chromatographic identity test for cefprozil.
436.369 Thin layer chromatography test for free *N*-isobutylpiperidone content in rifabutin.
436.370 Spectrophotometric identity test for rifabutin capsules.

Subpart G—Chemical Tests for Nonantibiotic Active Ingredients

436.400 Thin layer chromatographic identity test for iodochlorhydroxyquin.

Subpart H—Tests for Specific Antibiotic Dosage Forms

436.500 Penicillin in oil and wax.
436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.
436.504 Penicillin-bacitracin ointment.
436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.
436.506 Benzathine penicillin G and buffered crystalline penicillin for aqueous injection.
436.507 Benzathine-procaine-buffered crystalline penicillins for aqueous injection.
436.508 Penicillin - bacitracin - neomycin ointment; penicillin-bacitracin-neomycin in oil.
436.509 Procaine penicillin-streptomycin-polymyxin in oil; procaine penicillin-dihydrostreptomycin-polymyxin in oil; procaine penicillin-streptomycin-polymyxin ointment; procaine penicillin-dihydrostreptomycin-polymyxin ointment.
436.510 Penicillin - streptomycin - erythromycin ointment; penicillin-dihydrostreptomycin - erythromycin ointment.
436.511 Penicillin-streptomycin-bacitracin methylene disalicylate-neomycin ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate-neomycin ointment.
436.512 Procaine penicillin G-novobiocin-neomycin-dihydrostreptomycin in oil.

436.513 Chlortetracycline troches; tetracycline hydrochloride troches.
436.514 Chlortetracycline hydrochloride powder topical; tetracycline hydrochloride powder topical.
436.515 Capsules tetracycline and oleandomycin phosphate; capsules tetracycline and troleandomycin; capsules tetracycline hydrochloride and oleandomycin phosphate; capsules tetracycline hydrochloride and troleandomycin.
436.516 Tetracycline-neomycin complex powder topical; tetracycline hydrochloride-neomycin sulfate powder topical.
436.517 Bacitracin-neomycin tablets; zinc bacitracin-neomycin tablets; bacitracin methylene disalicylate-neomycin tablets.
436.542 Acid resistance/dissolution test for enteric-coated erythromycin pellets.
436.543 Acid resistance test for pellet-filled doxycycline hyclate capsules.
436.544 Dissolution test for pellet-filled doxycycline hyclate capsules.
436.545 Acid resistance test for erythromycin particles in tablets.

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SOURCE: 39 FR 18944, May 30, 1974, unless otherwise noted.

Subpart A—Definitions; Interpretations; Requirements

§ 436.1 Sterility requirements of items packaged with sterile antibiotic drugs.

(a) *Diluents packaged in combination with sterile antibiotic drugs.* If a sterile antibiotic drug is packaged in combination with an immediate container of a diluent, the immediate container of diluent shall be sterile when tested by the method prescribed in § 436.20(e)(1).

(b) *Dispensers packaged in combination with sterile antibiotic drugs.* If a sterile antibiotic drug is packaged in combination with a dispenser, such dispenser shall be sterile when tested by the method prescribed in § 436.20(e)(1).

[39 FR 18944, May 30, 1974, as amended at 41 FR 46852, Oct. 26, 1976]

§ 436.2 Alternative assay methods.

Alternative assay methods (including automated procedures) employing the same basic chemistry or microbiology as the official methods described in this part and in the individual monographs of this chapter may be used, provided the results obtained are of equivalent accuracy. However, only the results obtained from the official methods designated in the individual monographs are conclusive.

Subpart B—Sterility Test Methods

§ 436.20 Sterility test methods and procedures.

(a) *Laboratory facilities.* The test must be performed using aseptic techniques in an area as free from contamination as is possible to achieve. Testing should not be conducted under direct exposure to ultraviolet light or in areas under aerosol treatment. Environmental tests to assess the suitability of testing conditions should be made frequently enough to assure the validity of test results.

(b) *Equipment and reagents*—(1) *Bacterial membrane filter.* The filter has a nominal porosity of 0.45 micron±0.02 micron, a diameter of approximately 47 millimeters, and a flowrate of 55 milliliters to 75 milliliters of distilled water passing each square centimeter of filter area per minute with a differential pressure of 70 centimeters of mercury at 25° C.

(2) *Penicillinase solutions.* When the amount of penicillinase to be used is specified in terms of Levy units, use a penicillinase solution standardized in terms of Levy units. One Levy unit of penicillinase inactivates 59.3 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

(c) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. In lieu of preparing the media from the individual ingredients, they may be made from dehydrated mixtures which, when reconstituted with distilled water, have the same or equivalent composition as such media and have growth-promoting buffering, and oxygen tension-controlling properties equal to or better than such media. The pH of each medium should be adjusted with 2*N* hydrochloric acid or sodium hydroxide

before sterilization, so that after sterilization and the addition of the penicillinase, if necessary, the pH will fall within the specified range. Dispense 90±10 milliliter quantities of the liquid media into individual test tubes (38 millimeters x 200 millimeters). Close the tubes with suitable closures, and sterilize in an autoclave at 121° C. for 20 minutes. The autoclave temperature should be reached within 10 minutes. After sterilization, cool the medium at once to approximately 25° C. and store at 20° C. to 30° C. The sterility of each lot of tubes of liquid medium may be confirmed by incubating an adequate number of tubes as described in the test procedures in paragraph (e) of this section.

(1) *Medium A.* Use U.S.P. fluid thioglycolate medium I.

(2) *Medium B.* Use U.S.P. fluid thioglycolate medium I, with sufficient sterile penicillinase added to inactivate the penicillin activity in the sample under test. The penicillinase must be added to individual tubes of sterile medium A, using aseptic technique. Prior to use, or at the time of the test, a representative number of the tubes containing added penicillinase are incubated at 30° –32° C. for 24 hours to 48 hours, and are examined for sterility. If the sample contains penicillin as the only antibiotic, the ability of the penicillinase to inactivate all the penicillin in the sample under test is checked as follows: Add to one test tube of medium B the proper amount of penicillin from one of the individual containers under test. Then add 1.0 milliliter of a 1:1.000 dilution of an 18–24 hour culture of *Staphylococcus aureus* (American Type Culture Collection 6538–P)[1] in medium A. Typical microbial growth must be observable after 24 hours incubation at 30°–32° C. If the sample contains a mixture of penicillin plus some other antibiotic or antibacterial agent the ability of the penicillinase to inactivate all the penicillin in the sample is not tested directly on the sample under test, but is determined separately, using an amount of penicillin alone equivalent to the amount of penicillin in the sample or by any other suitable method for standardizing the penicillin-inactivating power of the penicillinase preparation.

(3) *Medium C.* To each liter of medium A add 5.0 milliliters of polysorbate 80 before sterilization. To each tube of sterilized medium add sufficient sterile penicillinase, and proceed as directed for medium B.

(4) *Medium D.* To each liter of medium A add 5.0 milliliters of polysorbate 80 and sufficient 2*N* sodium hydroxide so that the pH will be 7.9±0.1 after sterilization. Then add sufficient sterile penicillinase to each tube and proceed as directed for medium B.

(5) *Medium E.* Use U.S.P. XVIII soybean-casein digest medium.

(6) *Medium F.* To each liter of medium E add 5.0 milliliters of polysorbate 80 before sterilization. To each tube of sterilized medium add sufficient sterile penicillinase to solubilize the penicillin in the sample to be tested.

(7) *Medium G.* Prepare as follows:

Peptic digest of animal tissue	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.
pH 6.6±0.1	

Suspend the powder in a liter of distilled water. Allow to stand for 5 minutes, then mix thoroughly. Boil for 1 or 2 minutes or until solution is complete. Dispense in suitable flasks and sterilize at 121° C. for 15 minutes. Aseptically pour approximately 25-milliliter quantities into sterile Petri dish bottoms measuring 20 millimeters x 100 millimeters. Cover plates with sterile porcelain tops, glazed on the outside. Allow plates to stand at room temperature for 48 hours prior to use as a control on the sterility of the plates.

(8) *Medium H.* Prepare, sterilize, and dispense as described for medium G, except as follows:

Dextrose	40.0 gm.
Peptic digest of animal tissue	10.0 gm.
Agar	15.0 gm.
Distilled water q.s	1,000.0 ml.
pH 5.6±0.1 after sterilization	

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(9) *Medium I.* To each liter of Medium A add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol.

(10) *Medium J.* To each liter of Medium E add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol.

(11) *Medium K.* (*Rinse medium*). Prepare as follows:

Peptic digest of animal tissue	5.0 gm.
Beef extract	3.0 gm.
p-tert-octylphenoxy polyethoxyethanol	10.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.9±0.2 after sterilization

(12) *Medium L.* To each liter of Medium A add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol and approximately 10,000 Levy units of penicillinase.

(13) *Medium M.* To each liter of Medium E add 1 milliliter of *p-tert*-octylphenoxy polyethoxyethanol and approximately 10,000 Levy units of penicillinase.

(14) *Medium N:*

Pancreatic digest of casein	15.0 gm.
Peptic digest of soybean meal	5.0 gm.
Sodium chloride	5.0 gm.
Agar	15.0 gm.
Water	1,000.0 ml.

pH 7.3±0.2 after sterilization

(d) *Diluting fluids*—(1) *Diluting fluid A.* Dissolve 1 gram of U.S.P. peptic digest of animal tissue or equivalent in sufficient distilled water to make 1,000 milliliters. Dispense in flasks and sterilize as described in paragraph (c) of this section. Final pH=7.1±0.1.

(2) *Diluting fluid B.* To each liter of diluting fluid A add 5.0 milliliters of polysorbate 80 before sterilization.

(3) *Diluting fluid C.* To each liter of diluting fluid A add 0.5 gram of sodium thioglycollate, and adjust with NaOH so that after sterilization the final pH will be pH 6.6±0.6. Dispense in flasks and sterilize as described in paragraph (c) of this section.

(4) *Diluting fluid D.* To each liter of diluting fluid A add 1 milliliter of *p-tert*- octylphenoxy polyethoxyethanol. Dispense in flasks and sterilize as described in paragraph (c) of this section. Final pH=7.1±10.1.

(5) *Diluting fluid E.* Use isopropyl myristate that is sterile and that has a water-extract pH of 5.5 or greater. Determine the water-extract pH of a portion of the isopropyl myristate as follows: Place 100 milliliters of the isopropyl myristate sample and 10 milliliters of distilled water into a centrifuge bottle of approximately 250 milliliters capacity and seal the bottle tightly. Place the centrifuge bottle on a shaker so that its longest dimension is oriented in the direction of shaker movement and shake at 250 cycles per minute for 1 hour. Centrifuge the bottle at 1,800 revolutions per minute for 20 minutes. With a suitable vacuum system, remove and discard the upper layer; then pipet 5 milliliters of the lower water layer into a beaker and determine the pH using a standardized pH meter. If the water-extract pH is less than 5.5, pass the isopropyl myristate through a glass column packed with basic aluminum oxide, activity grade No. 1. Determine the water-extract pH of a portion of the isopropyl myristate that has been passed through the aluminum oxide column. Sterilize isopropyl myristate by filtration through a 0.22-micron membrane filter and aseptically dispense 100-milliliter portions into sterile 250-milliliter flasks.

(6) *Diluting fluid F.* To each liter of diluting fluid A add 20 grams of disodium edetate, and adjust with NaOH so that after sterilization the final pH will be 7.1±0.1. Dispense in flasks and sterilize as described in paragraph (c) of this section.

(7) *Diluting fluid G.* To each liter of sterile diluting fluid A add 10 grams of sterile *L*-lysine.

(8) *Diluting fluid H.* To each liter of diluting fluid A add 10 grams of sodium bicarbonate before sterilization.

(9) *Diluting fluid I.* To each liter of diluting fluid A add 23.4 grams of sterile L-arginine base.

(10) *Diluting fluid J.* Sterilize 2.0 grams of anhydrous sodium carbonate by dry-heating at 180° C for 2 hours. Dissolve in 100 milliliters of diluting fluid A just prior to use.

(e) *Conduct of test*—(1) *Bacterial membrane filter method*—(i) *Sample preparation*—(*a*) Antibiotic drug. From each of 20 immediate containers, aseptically transfer approximately 300 milligrams of solids if it is not a liquid drug, or 1 milliliter by volume if it is a liquid drug, or the entire contents if the container contains less than these amounts; except that if it is a liquid

drug containing penicillin in a concentration greater than 300,000 units per milliliter, use the volume that contains 300,000 units, into a sterile 500-milliliter Erlenmeyer flask containing approximately 200 milliliters of diluting fluid A. (If it is a composite sample packaged in one immediate container in accordance with the requirements of § 431.5(b) of this chapter, transfer the entire contents, or approximately 6 grams, into the Erlenmeyer flask.) Stopper the flask and swirl to dissolve the drug. As soon as the sample has completely dissolved, proceed as directed in paragraph (e)(1)(ii) of this section. If the pooled portions from 20 containers will not dissolve completely in 200 milliliters of diluting fluid or will not filter rapidly, 400 milliliters of diluting fluid may be used or two separate tests may be performed using a pool of 10 containers for each test.

(*b*) Diluent packaged in combination with a sterile drug. Using the entire contents from each of 20 immediate containers, proceed as directed in paragraph (e)(1)(ii) of this section.

(*c*) Sterile dispensers packaged in combination with a sterile drug. Prepare 20 clean, empty containers of approximately the same size as those in which the sterile antibiotic drug is packaged. To each container add diluting fluid A in a volume approximately the same as that of the sterile drug when it is prepared for dispensing. Cap the containers, sterilize by autoclaving at 121° C. for 20 minutes, and then allow to cool to room temperature. Aseptically open each dispenser package and remove each dispenser in turn. Use each aseptically to remove 1 milliliter of the fluid from a separate sterile container prepared as described above. Aseptically transfer the fluid to a 500-milliliter Erlenmeyer flask containing approximately 200 milliliters of diluting fluid A. Stopper the flask and proceed as directed in paragraph (e)(1)(ii) of this section.

(ii) *Test procedure.* Aseptically filter the solution through a bacteriological membrane filter. All air entering the filtering system is filtered through air filters capable of removing microorganisms. Filter three 100-milliliter quantities of diluting fluid A through the membrane. For the penicillin and cephalosporin classes of antibiotics, add sufficient penicillinase to diluting fluid A to inactivate the residual antibiotic activity on the membrane after filtration. By means of a sterile circular blade, paper punch, or any other suitable sterile device, cut a circular portion (approximately 17.5 millimeters in diameter) from the center of the filtering area. Transfer the cut center area to a sterile 38 by 200 millimeter (outside dimensions) test tube containing 90±10 milliliters of sterile medium A. Incubate the tube for 7 days at 30°–32° C. Using sterile forceps, transfer the remaining outer portion of the membrane into a second similar tube containing 90±10 milliliters of medium E. Incubate the second tube for 7 days at 22°–25° C.

(2) *Direct method.* From each of 20 immediate containers, transfer approximately 300 milligrams of solids if it is not a liquid drug, or 1 milliliter by volume if it is a liquid drug, or the entire contents if it contains less than these amounts, except if it is a liquid drug containing penicillin in a concentration greater than 300,000 units per milliliter use that volume that contains 300,000 units, into individual sterile test tubes (38 millimeters × 200 millimeters) containing 90±10 milliliters of medium A. Incubate all tubes at 30° C. to 32° C. for 7 days. Gently agitate the tubes every 1 to 3 days or until complete solubilization occurs. At intervals, examine all tubes for visible growth. If growth is observed in any tube, confirm by microscopic examination. From each of the same 20 immediate containers, transfer a second portion (equivalent to that portion initially transferred to the tubes containing medium A) to individual sterile test tubes (38 millimeters × 200 millimeters) containing 90±10 milliliters of medium E, except when each container does not have sufficient material to provide for the two similar-size portions, obtain the second portion from 20 additional immediate containers. Incubate all tubes at 22° C. to 25° C. for 7 days. Gently agitate the tubes every 1 to 3 days or until complete solubilization occurs. At intervals, examine all tubes for visible growth. If growth is observed in any tube, confirm by microscopic examination.

(3) *Bacterial membrane filter method for ophthalmic ointments*—(i) *Ointments that do not contain penicillin.* From each of 10 immediate containers aseptically transfer 0.1 gram of the product into a sterile 250-milliliter flask containing 100 milliliters of diluting fluid E which has previously been heated to a temperature of 47° C. Repeat the process, using 10 additional containers. Swirl both of the flasks to dissolve the ointment. Immediately aseptically filter each solution through a separate bacteriological membrane filter previously moistened with approximately 0.2 milliliter of medium K. Filter all air entering the system through air filters capable of removing microorganisms. Remove any residual antibiotic from the membranes by rinsing each filter five times with 100 milliliters of medium K. The membranes should be covered with fluid throughout each step of the filtration procedure until the end of the last filtering step. By means of a sterile circular blade, paper punch, or other suitable sterile device, cut a circular portion (approximately 17.5 millimeters in diameter) from the center of the filtering area of each membrane. Transfer the center portion of the filtering area of each filter to a sterile test tube 38 millimeters × 200 millimeters (outside dimensions) containing 90 milliliters±10 milliliters of sterile medium I. Incubate the tube for 7 days at 30° C. to 32° C. Using sterile forceps transfer the outer portion of each filter to a similar test tube containing 90 milliliters ±10 milliliters of sterile medium J. Incubate this tube for 7 days at 22° C. to 25° C.

(ii) *Ointments containing penicillin.* Proceed as directed in paragraph (e)(3)(i) of this section, except in lieu of sterile medium I use sterile medium L for the center portion of the filtering area of each filter and in lieu of sterile medium J use sterile medium M for the remaining outer portion of each filter.

(f) *Evaluation of results*—(1) *Bacterial membrane-filter method.* The batch, or the part of the batch represented by a particular filling operation meets the requirements of the test if no sample tube shows growth. If growth is observed in any sample tube, run a second test in the appropriate medium, except perform it in duplicate, using 40 immediate containers. If in the original test, growth is observed in only one of the two media, test both portions of the cut filter membrane by placing each into a separate tube of the same medium. The batch meets the requirements if no tube on the second test shows growth. If growth is observed in any of the control tubes as well as in the sample tubes in either the original or the second test such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained in the first and second tests may not be valid. In such instances, the batch is satisfactory if on the final test no tube shows growth.

(2) *Direct method.* The batch, or the part of the batch represented by a particular filling operation, meets the requirements of the test if no tube shows growth after incubation. If growth is observed in any sample tube, run a second test in the appropriate medium using 40 immediate containers. The batch is satisfactory if, on the second test, no tube shows growth. If growth is observed in any of the control tubes (except inoculated tubes, if the sample is penicillin) as well as in the sample tubes in either the original or the second test, such test is invalid and must be performed again. In any event, further tests may be justified if there is sufficient reason to believe that the results obtained on the first and second tests may not be valid. In such instances the batch is satisfactory if in the final test no tube shows growth.

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Subpart C—Biological Test Methods

§ 436.31 Equipment and diluents for use in biological testing.

(a) *Equipment*—(1) *Temperature-measuring devices.* Use an accurate clinical

thermometer or any other temperature-measuring device of equal sensitivity that has been tested to determine the time necessary to reach the maximum reading.

(2) *Pyrogen-free glassware.* Render all glassware free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(3) *Pyrogen-free syringes and needles.* Render all syringes and needles free from pyrogens by heating at 250° C. for not less than 30 minutes or by any other suitable method.

(4) *Pyrogen-free sodium chloride.* Heat sodium chloride for not less than 2 hours at 200° C.

(5) *Pyrogen-free sodium carbonate.* Heat anhydrous sodium carbonate for not less than 4 hours at 170° C.

(b) *Diluents.* (1) Diluent 1 (pyrogen-free water): Prepare pyrogen-free water by collecting freshly distilled water and sterilizing it in an autoclave at 121° C. for not less than 20 minutes. Pyrogen-free water meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2). In testing water for the absence of pyrogens, the aliquot to be tested is made isotonic by the addition of pyrogen-free sodium chloride.

(2) Diluent 2 (pyrogen-free saline solution): Prepare an isotonic solution of sodium chloride by dissolving 9.0 grams of pyrogen-free sodium chloride (prepared as described in § 436.31(a)(4)) in pyrogen-free, distilled water (diluent 1) to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for not less than 20 minutes. Pyrogen-free saline solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 10 milliliters per kilogram are administered as described in § 436.32(a)(2).

(3) Diluent 3 (sterile distilled water): Prepare freshly distilled water. Sterilize in an autoclave at 121° C. for 20 minutes.

(4) Diluent 4 (sterile saline solution): Dissolve 9.0 grams of sodium chloride in distilled water to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for 20 minutes.

(5) Diluent 5 (10 percent gum acacia): Dissolve 10 grams of gum acacia in approximately 50 milliliters of distilled water. Allow to stand overnight at room temperature and dilute to 100 milliliters with distilled water. Filter through cotton. Store under refrigeration.

(6) Diluent 6 (0.5 percent gum acacia in distilled water). 11132

(7) Diluent 7 (1.0N hydrochloric acid).

(8) Diluent 8 (0.1N hydrochloric acid).

(9) Diluent 9 (0.05N sodium hydroxide).

(10) Diluent 10 (1 percent U.S.P. methylcellulose (4,000 centipoises) solution): Dissolve 1 gram of U.S.P. methylcellulose (4,000 centipoises) in 100 milliliters of distilled water. Allow to stand overnight at room temperature or until solution is complete. Store under refrigeration.

(11) Diluent 11 (0.12*N* sodium hydroxide).

(12) Diluent 12 (0.5 percent methylcellulose (4,000 centipoises) in distilled water). Proceed as directed in paragraph (b)(10) of this section, except use 0.5 gram of methylcellulose (4,000 centipoises).

(13) Diluent 13 (pyrogen-free sodium carbonate solution). Dissolve 25.6 grams of anhydrous pyrogen-free sodium carbonate (prepared as described in paragraph (a)(5) of this section) in 1,000 milliliters pyrogen-free, distilled water (diluent 1). Pyrogen-free, sodium carbonate solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 1.0 milliliter per kilogram is administered as described in § 436.32(a)(2).

(14) Diluent 14 (0.07M sterile sodium carbonate solution). Dissolve 7.3 grams of sodium carbonate in distilled water to make 1,000 milliliters. Sterilize in an autoclave at 121° C. for 20 minutes.

(15) Diluent 15 (pyrogen-free sodium carbonate solution): Dissolve 9.9 grams of anhydrous pyrogen-free sodium carbonate (prepared as directed in paragraph (a)(5) of this section) in 1,000 milliliters of pyrogen-free, distilled water (diluent 1). Pyrogen-free sodium carbonate solution meets the requirements for the absence of pyrogens as described in § 436.32(a)(3) when 1.0 milliliter per kilogram is administered as described in § 436.32(a)(2).

(16) Diluent 16 (0.13*M* sterile pyrogen-free sodium carbonate solution). Dissolve 14.0 grams of anhydrous pyrogen-free sodium carbonate (prepared as described in paragraph (a)(5) of this section) in 1,000 milliliters pyrogen-free, distilled water. Sterilize in an autoclave at 121 °C for 20 minutes.

[39 FR 18944, May 30, 1974, as amended at 40 FR 51625, Nov. 6, 1975; 50 FR 48397, Nov. 25, 1985; 53 FR 13401, Apr. 25, 1988]

§ 436.32 Pyrogen test.

(a) *Method 1*—(1) *Test animal.* Use healthy, mature rabbits weighing not less than 1,800 grams each that have maintained their weight on an antibiotic-free diet for at least 1 week under the environmental conditions specified in this section. House the animals individually in an area of uniform temperature (±3° C.) and free from disturbances likely to excite them. Do not use animals for pyrogen tests more frequently than once every 48 hours or prior to 2 weeks following their having been given a test sample that was adjudged pyrogenic. Before using an animal that has not been used for a test during the previous 2 weeks, condition it 1 to 3 days prior to pyrogen testing by conducting a sham test as directed in paragraph (a)(2) of this section, omitting the injection.

(2) *Procedure.* Using equipment and diluents described in § 436.31, as necessary, perform the test in an area where the animals are housed or under similar environmental conditions. On the day of the test: Withhold all food from the animals being used until after completion of the test, except that access to water may be allowed; and determine the ''control temperature'' of each animal by inserting the temperature-measuring device into the rectum of the test animal to a depth of not less than 7.5 centimeters and allowing sufficient time to reach a maximum temperature, as previously determined, before taking the reading. In any one test use only those animals whose control temperatures do not deviate by more than 1° C. from each other and do not use any animal with a temperature exceeding 39.8° C. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated. If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling. Warm the product to be tested to approximately 37° C. Dilute the sample with sterile, pyrogen-free saline (prepared as described in § 436.31(b)(2)) to the appropriate concentration specified in the individual section for each antibiotic to be tested. Inject a test dose of 1 milliliter of the diluted sample per kilogram of rabbit weight into an ear vein of each of three rabbits within 30 minutes subsequent to the control temperature reading. Record the temperature at 1, 2, and 3 hours subsequent to the injection.

(3) *Evaluation.* If no rabbit shows an individual rise in temperature of 0.6° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 1.4° C., the sample meets the requirements for the absence of pyrogens. If one or two rabbits show a temperature rise of 0.6° C. or more, or if the sum of the temperature rises exceeds 1.4° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.6° C. or more, and if the sum of the eight temperature rises does not exceed 3.7° C., the sample meets the requirements for the absence of pyrogens.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1).

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dilute the sample with pyrogen-free water (diluent 1) and inject a test dose of 0.5 milliliter of the diluted sample per kilogram of rabbit weight.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dilute sample with 0.05*N* sodium hydroxide (diluent 9).

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dilute sample with sodium carbonate solution (diluent 13).

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except inject a test dose of 2.0 milliliters of the diluted sample per kilogram of rabbit weight.

(i) *Method 9.* Proceed as directed in paragraph (a) of this section, except dilute sample with pyrogen-free sodium carbonate solution (diluent 15).

(j) *Method 10.* Proceed as directed in paragraph (a) of this section, except dilute the sample with sodium carbonate solution (diluent 16).

[39 FR 18944, May 30, 1974, as amended at 40 FR 51625, Nov. 6, 1975; 45 FR 22921, Apr. 4, 1980; 50 FR 48397, Nov. 25, 1985; 53 FR 13401, Apr. 25, 1988]

§ 436.35 Depressor substances test.

Proceed as directed in the USP XX depressor substances test. Prepare the sample test solution as follows: For each antibiotic listed in the table below, select the appropriate diluent and test dose (concentration and volume). If the product is packaged for dispensing and is in a combination package with a container of diluent, dilute the product as directed in the labeling.

Antibiotic	Diluent [1]	Concentration of test solution [2]	Volume of test solution to be injected [3]
Bleomycin sulfate	4	[4] 0.5	1.0
Capreomycin sulfate	4	3.0	1.0
Chlortetracycline hydrochloride	3	5.0	.6
Clindamycin phosphate	4	5.0	1.0
Daunorubicin hydrochloride	4	1.5	1.0
Dihydrostreptomycin sulfate	4	3.0	1.0
Doxorubicin hydrochloride	4	1.5	1.0
Doxycycline hyclate	4	5.0	1.0
Lincomycin hydrochloride monohydrate	4	3.0	1.0
Minocycline hydrochloride	4	5.0	.6
Plicamycin	3	0.050	1.0
Mitomycin	4	0.050	1.0
Oxytetracycline [5]	3	5.0	.6
Oxytetracycline hydrochloride	4	5.0	.6
Rolitetracycline	4	5.0	.6
Rolitetracycline nitrate	4	5.0	.6
Sodium colistimethate	4	3.0	1.6
Spectinomycin hydrochloride	4	15.0	1.0
Streptomycin sulfate	4	3.0	1.0
Tetracycline hydrochloride	4	5.0	.6
Tetracycline phosphate [5]	3	5.0	.6
Vidarabine monohydrate [5]	4	1.0	1.0

[1] Diluent number as listed in sec. 436.31(b).
[2] Milligrams of activity per milliliter.
[3] Milliliters per kilogram of body weight.
[4] The concentration of the test solution is expressed in units per milliliter in lieu of milligrams of activity per milliliter.
[5] To prepare the test solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

[46 FR 60568, Dec. 11, 1981, as amended at 46 FR 61071, Dec. 15, 1981; 49 FR 5096, Feb. 10, 1984]

Subpart D—Microbiological Assay Methods

§ 436.100 Laboratory equipment.

Equipment should be selected which is adequate for its intended use and should be thoroughly cleansed after each use to remove any antibiotic residues. The equipment should be kept covered when not in use. Clean glassware intended for holding and transferring the test organisms should be sterilized in a hot air oven at 200–220° C. for 2 hours. Volumetric flasks, pipettes, or accurately calibrated diluting devices should be used when diluting standard and sample solutions.

(a) *Microbiological agar diffusion assay*—(1) *Cylinders.* Use stainless steel cylinders with an outside diameter of 8 millimeters (±0.1 millimeter), an inside diameter of 6 millimeters (±0.1 millimeter), and a length of 10 millimeters (±0.1 millimeter).

(2) *Plates.* Plastic or glass Petri dishes may be used, having dimensions of 20 by 100 millimeters. Covers should be of suitable material.

(b) *Microbiological turbidimetric assay*—(1) *Tubes.* Tubes which give satisfactory results and have uniform length and diameter should be used. If reusable tubes are employed, care must be taken to remove not only all antibiotic residues from the previous test but also all traces of cleaning solution.

(2) *Colorimeter.* Use a suitable photoelectric colorimeter at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear,

uninoculated broth prepared as described in the applicable method for the antibiotic being assayed.

[39 FR 18944, May 30, 1974, as amended at 41 FR 34743, Aug. 17, 1976]

§ 436.101 Solutions.

(a) Antibiotic assay solutions are prepared as follows (solution numbers 1, 2, 3, 4, and 6 correspond to those used in ''Assay Methods of Antibiotics,'' D. C. Grove and W. A. Randall, Medical Encyclopedia, Inc., New York, N.Y. (1955), p. 222), which is incorporated by reference. Copies are available from the Medical Encyclopedia Inc., 30 East 60th St., New York, NY 11220, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

(1) *Solution 1* (*1 percent potassium phosphate buffer, pH 6.0*).

Dibasic potassium phosphate: 2.0 gm.
Monobasic potassium phosphate: 8.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(2) *Solution 2* (*citrate buffer solution pH 6.3*).

Citric acid: 13.2 gm.
Sodium hydroxide: 7.06 gm.
Sodium citrate: 97.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 10 percent citric acid solution or 10*N* sodium hydroxide to yield pH 6.2 to 6.4 after sterilization.

(3) *Solution 3* (*0.1M potassium phosphate buffer, pH 8.0*).

Dibasic potassium phosphate: 16.73 gm.
Monobasic potassium phosphate: 0.523 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 7.9 to 8.1 after sterilization.

(4) *Solution 4*(*0.1M potassium phosphate buffer, pH 4.5*).

Monobasic potassium phosphate: 13.6 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 4.45 to 4.55 after sterilization.

(5) [Reserved]

(6) *Solution 6* (*10 percent potassium phosphate buffer, pH 6.0*).

Dibasic potassium phosphate: 20.0 gm.
Monobasic potassium phosphate: 80.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 5.95 to 6.05 after sterilization.

(7)–(9) [Reserved]

(10) *Solution 10* (*0.2M potassium phosphate buffer, pH 10.5*).

Dibasic potassium phosphate: 35.0 gm.
10 N potassium hydroxide: 2.0 ml.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 10.4 to 10.6 after sterilization.

(11) *Solution 11* (*10 percent potassium phosphate buffer, pH 2.5*).

Monobasic potassium phosphate: 100.0 gm.
Concentrated hydrochloric acid: 0.2 ml. (approximately).
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 2.0 to 2.8 after sterilization.

(12) *Solution 12* (*10 percent potassium phosphate buffer, pH 7.0*).

Monobasic potassium phosphate: 100.0 gm.
Distilled water, q.s: 1,000.0 ml.

Adjust with 18*N* phosphoric acid or 10*N* potassium hydroxide to yield a pH 6.95 to 7.05 after sterilization.

(13) *Solution 13* (*0.01N methanolic hydrochloric acid*).

1.0N hydrochloric acid: 10.0 ml.
Methyl alcohol, q.s: 1,000.0 ml.

(14) *Solution 14* (*2 percent sodium bicarbonate solution*).

Sodium bicarbonate: 20.0 gm.
Distilled water, q.s: 1,000.0 ml.

Prepare daily.

(15) *Solution 15* (*80 percent isopropyl alcohol solution*).

Isopropyl alcohol: 800.0 ml.
Distilled water, q.s: 1,000.0 ml.

(16) *Solution 16* (*0.1 M potassium phosphate buffer, pH 7.0*).

Dibasic potassium phosphate: 13.6 gm.
Monobasic potassium phosphate: 4.0 gm.
Distilled water, q.s.: 1,000.0 ml.

Adjust with 18 *N* phosphoric acid or 10 *N* potassium hydroxide to yield a pH 6.8 to 7.2 after sterilization.

(17) *Solution 17* (*5 percent methyl alcohol in 1 percent potassium phosphate buffer, pH 6.0*).

Methyl alcohol: 50.0 ml.
1 percent potassium phosphate buffer, pH 6.0, q.s.: 1,000.0 ml.

(18) *Solution 18 (0.054M sodium phosphate buffer, pH 6.9).*

Sodium dihydrogen phosphate monohydrate: 3.97 gm.
Disodium hydrogen phosphate anhydrous: 3.55 gm.
Distilled water, q.s.: 1,000.0 mL.

[39 FR 18944, May 30, 1974, as amended at 40 FR 52004, Nov. 7, 1975; 45 FR 75194, Nov. 14, 1980; 47 FR 9396, Mar. 5, 1982]

§ 436.102 Culture media.

(a) *Ingredients.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F. In lieu of preparing the media from the individual ingredients specified, they may be made from dehydrated mixtures that, when reconstituted with distilled water, have the same composition as such media. Minor modifications of the individual ingredients specified in this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(b) *Description of media.* Medium numbers 1, 2, 3, 4, 5, 8, 9, 10, 11, and 13 correspond to those used in ''Assay Methods of Antibiotics,'' D. C. Grove and W. A. Randall, Medical Encyclopedia, Inc., New York, N.Y. (1955) p. 220, which is incorporated by reference. Copies are available from Medical Encyclopedia Inc., 30 East 60th St., New York, NY, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC. Medium numbers 18 through 21 correspond to those used in ''Outline of Details for Official Microbiological Assays of Antibiotics,'' A. Kirshbaum and B. Arret, ''Journal of Pharmaceutical Sciences,'' vol. 56, No. 4, April 1967, p. 512, which is incorporated by reference. Copies are available from the American Pharmaceutical Association, 2215 Constitution Ave. NW., Washington, DC 20037, or available for inspection at the Office of the Federal Register (see address in this paragraph).

(1) *Medium 1.*

Peptone: 6.0 gm.
Pancreatic digest of casein: 4.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Dextrose: 1.0 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(2) *Medium 2.*

Peptone: 6.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(3) *Medium 3.*

Peptone: 5.0 gm.
Yeast extract: 1.5 gm.
Beef extract: 1.5 gm.
Sodium chloride: 3.5 gm.
Dextrose: 1.0 gm.
Dipotassium phosphate: 3.68 gm.
Potassium dihydrogen phosphate: 1.32 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.95 to 7.05 after sterilization.

(4) *Medium 4.*

Peptone: 6.0 gm.
Yeast extract: 3.0 gm.
Beef extract: 1.5 gm.
Dextrose: 1.0 gm.
Agar: 15.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.5 to 6.6 after sterilization.

(5) *Medium 5.* Medium 5 is the same as medium 2, except adjust the final pH to 7.8 to 8.0 after sterilization.

(6)–(7) [Reserved]

(8) *Medium 8.* Medium 8 is the same as medium 2, except adjust the final pH to 5.8 to 6.0 after sterilization.

(9) *Medium 9.*

Pancreatic digest of casein: 17.0 gm.
Papaic digest of soybean: 3.0 gm.
Sodium chloride: 5.0 gm.
Dipotassium phosphate: 2.5 gm.
Dextrose: 2.5 gm.
Agar: 20.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 7.2 to 7.3 after sterilization.

(10) *Medium 10.* Medium 10 is the same as medium 9, except:

Agar: 12.0 gm.
Polysorbate 80 (add polysorbate 80 after boiling the medium to dissolve the agar): 10.0 ml.
pH 7.2 to 7.3 after sterilization.

(11) *Medium 11.* Medium 11 is the same as medium 1, except adjust the final pH to 7.8 to 8.0 after sterilization.

(12) [Reserved]

(13) *Medium 13.*

Peptone: 10.0 gm.

Dextrose: 20.0 gm.
Distilled water, q.s: 1,000.0 ml.
pH 5.6 to 5.7 after sterilization.

(14)–(18) [Reserved]

(19) *Medium 19.*

Peptone: 9.4 gm.
Yeast extract: 4.7 gm.
Beef extract: 2.4 gm.
Sodium chloride: 10.0 gm.
Dextrose: 10.0 gm.
Agar: 23.5 gm.
Distilled water, q.s: 1,000.0 ml.
pH 6.0 to 6.2 after sterilization.

(20)–(31) [Reserved]

(32) *Medium 32.* Prepare as medium 1, except add 300 milligrams of hydrated manganese sulfate ($MnSO_4 \cdot H_2O$) to each liter of medium.

(33) *Medium 33.* Use medium 1, sterilized and cooled to 50° C. Aseptically add sufficient sterile sodium novobiocin solution to give a final concentration of 10 micrograms of novobiocin activity per milliliter of medium. Sterile sodium novobiocin solution is prepared by filtering a solution containing 2.5 milligrams of novobiocin per milliliter of distilled water through a membrane filter of 0.22–micron porosity.

(34) *Medium 34.*

Glycerol: 10.0 gm.
Peptone: 10.0 gm.
Beef extract: 10.0 gm.
Sodium chloride: 3.0 gm.
Distilled water, q.s.: 1,000.0 ml.
pH 7.0 after sterilization.

(35) *Medium 35.* Same as medium 34, except add 17.0 grams of agar to each liter of medium.

(36) *Medium 36.*

Pancreatic digest of casein	15.0 gm.
Papaic digest of soybean	5.0 gm.
Sodium chloride	5.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.
pH 7.3 after sterilization	.....

(37) *Medium 37.*

Pancreatic digest of casein: 17.0 gm.
Soybean peptone: 3.0 gm.
Dextrose: 2.5 gm.
Sodium chloride: 5.0 gm.
Dipotassium phosphate: 2.5 gm.
Distilled water, q.s.: 1,000.0 ml.
pH 7.3 after sterilization.

(38) *Medium 38.*

Peptone: 15.0 gm.
Papaic digest of soybean meal: 5.0 gm.
Sodium chloride: 4.0 gm.
Sodium sulfite: 0.2 gm.
L-cystine: 0.7 gm.
Dextrose: 5.5 gm.
Agar: 15.0 gm.
Distilled water, q.s.: 1,000.0 ml.
pH 7.0 after sterilization.

[39 FR 18944, May 30, 1974, as amended at 40 FR 52004, Nov. 7, 1975; 42 FR 14092, Mar. 15, 1977; 47 FR 9396, Mar. 5, 1982; 47 FR 22514, May 25, 1982]

§ 436.103 Test organisms.

(a) *Preparation of test organism suspensions.* For each test organism listed in the following table, select the media (as listed by medium number in § 436.102(b)), incubation period of the Roux bottle, suggested dilution factor, and suggested storage period for the particular test organism and proceed by the appropriate method described in paragraph (b) of this section. Test organism letters A through K, M, and N correspond to those used in ''Outline of Details for Official Microbiological Assays of Antibiotics,'' A. Kirshbaum and B. Arret, ''Journal of Pharmaceutical Sciences,'' Vol. 56, No. 4, p. 512 (April 1967), which is incorporated by reference. Copies are available from the American Pharmaceutical Association, 2215 Constitution Ave. NW., Washington, DC 20037, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

Test organisms	Method used	Medium used for the—		Incubation period of Roux bottle	Suggested dilution factor	Suggested storage period of suspensions under refrigeration
		Slants	Roux bottles			
Test organism A—*Staphylococcus aureus* (ATCC 6538P) [2].	1	1	1	24 hours	1:20	1 week.
Test organism B—*Micrococcus luteus* (ATCC 7468) [2].	1	1	1	24 hours	1:30	2 weeks.
Test organism C—*Micrococcus luteus* (ATCC 9341) [2].	1	1	1	24 hours	1:40	2 weeks.
Test organism D—*Staphyloccocus epidermidis* (ATCC 12228) [2].	1	1	1	24 hours	[1] 1:14	1 week.

Test organisms	Method used	Medium used for the—		Incubation period of Roux bottle	Suggested dilution factor	Suggested storage period of suspensions under refrigeration
		Slants	Roux bottles			
Test organism E—*Saccharomyces cerevisiae* (ATCC 9763)[2].	6	19	.....	.....	1:30	4 weeks.
	or					
	7	19	19	48 hours	1:30	4 weeks.
Test organism F—*Bordetella bronchiseptica* (ATCC 4617)[2].	1	1	1	24 hours	1:20	2 weeks.
Test organism G—*Bacillus cereus* var. *mycoides* (ATCC 11778)[2].	3	1	1	1 week	.....	6 months.
Test organism H—*Bacillus subtilis* (ATCC 6633)[2].	1	1	1	24 hours	.....	6 months.
	or					
	2	1	32	5 days	.....	6 months.
Test organism I—*Klebsiella pneumoniae* (ATCC 10031)[2].	1	1	1	24 hours	1:25	1 week.
Test organism J—*Escherichia coli* (ATCC 10536).	1	1	1	24 hours	1:20	2 weeks.
Test organism K—*Streptococcus faecium* (ATCC 10541)[2].	5	.....	.....	.....	.....	24 hours.
Test organism L—*Micrococcus luteus* (ATCC 10240)[2].	1	1	1	24 hours	1:35	4 weeks.
Test organism O—*Staphylococcus aureus,* resistant to novobiocin (ATCC 12692)[2].	1	33	33	24 hours	1:10	4 weeks.
Test organism T—*Saccharomyces cerevisiae* (ATCC 2601)[2].	7	19	19	48 hours	1:30	4 weeks.
Test organism V—*Micrococcusluteus,* resistant to dihydrostreptomycin (ATCC 10240A)[2].	1	1	1	48 hours	1:35	4 weeks.
Test organism W—*Pseudomonas aeruginosa* (ATCC 25619)[2].	1	1	1	24 hours	1:25	2 weeks.
Test organism X—*Mycobacterium smegmatis* (ATCC 607)..	8	36	.....	.....	.....	2 weeks.
Test organism Y—*Pseudomonas aeruginosa* (ATCC 29336)[2].	9	36	36	24 hours	1:50	1 week.

[1] If the antibiotic to be tested is paromomycin, the dilution factor is 1:25.
[2] Available from American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD. 20852.

(b) *Methods for preparation of test organism suspensions*—(1) *Method 1*—(i) *Preparation of suspension.* Maintain organisms on agar slants containing 10 milliliters of the appropriate medium. Incubate the slants at 32° C.–35° C. for 24 hours. Using 3 milliliters of sterile U.S.P. saline T.S., wash the growth from the agar slant onto a large agar surface, such as a Roux bottle, containing 250 milliliters of the appropriate medium. Spread the suspension of organisms over the entire surface of the Roux bottle with the aid of sterile glass beads. Incubate the Roux bottle at 32° C.–35° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline T.S.

(ii) *Standardization of suspension.* Determine the dilution factor that will give a 25–percent light transmission at a wavelength of 580 millimicrons using a suitable photoelectric colorimeter and a 13–millimeter diameter test tube as an absorption cell. It may be necessary to adjust the suspension. Determine the amount of suspension to be added to each 100 milliliters of agar or nutrient broth by the use of test plates or test broth. Store the test organism suspension under refrigeration.

(2) *Method 2.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of paragraph (b)(1)(ii) thereof, heat-shock and standardize the suspension as follows: Centrifuge and decant the supernatant liquid. Resuspend the sediment with 50 to 70 milliliters of sterile U.S.P. saline T.S. and heat the suspension for 30 minutes at 70° C. Use test plates to assure the viability of the spores and to determine the amount of spore suspension to be added to each 100 milliliters of agar. Maintain the spore suspension under refrigeration.

(3) *Method 3.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of paragraph (b)(1)(ii) thereof, heat-shock and standardize the suspension as follows: Heat the suspension for

30 minutes at 70° C. Wash the spore suspension three times with 25 to 50 milliliters of sterile distilled water. Resuspend the organisms in 50 to 70 milliliters of sterile distilled water and heat-shock again for 30 minutes at 70° C. Use test plates to assure the viability of the spores and to determine the amount of spore suspension to be added to each 100 milliliters of agar. Maintain the spore suspension under refrigeration.

(4) [Reserved]

(5) *Method 5.* Maintain the test organisms in 100–milliliter quantities of nutrient broth—Medium 3 as described in § 436.102(b)(3). For the test prepare a fresh subculture by transferring a loopful of the stock culture to 100 milliliters of the same nutrient broth and incubate for 16 to 18 hours at 37° C. Store this broth culture under refrigeration.

(6) *Method 6.* Maintain the test organisms on agar slants containing 10 milliliters of the medium specified in paragraph (a) of this section. Incubate the slants at 32° C.–35° C. for 24 hours. Inoculate 100 milliliters of nutrient broth—Medium 13 as described in § 436.102(b)(13). Incubate for 16 to 18 hours at 37° C. Proceed as directed in paragraph (b)(1)(ii) of this section.

(7) *Method 7.* Proceed as directed in paragraph (b)(1) of this section, except incubate the slants at 30° C. for 24 hours and incubate the Roux bottle at 30° C. for 48 hours.

(8) *Method 8.* Maintain organisms on agar slants containing 10 milliliters of the appropriate medium and transfer to a fresh slant about once a week. Incubate the slants at 37° C for 48 hours. Using 3 milliliters of sterile U.S.P. saline T.S., wash the growth from the agar slant into a 500–milliliter Erlenmeyer flask containing 100 milliliters of medium 34, as described in § 436.102(b) (34), and 50 grams of glass beads. Agitate the culture by rotation at a speed of 130 cycles per minute and a radius of 3.5 centimeters at 27° C for 5 days. Determine the amount of suspension to be added to each 100 milliliters of agar by the use of test plates. Store the test organism suspension under refrigeration.

(9) *Method 9.* Proceed as directed in paragraph (b)(1) of this section, except incubate the slant and Roux bottle at 37° C and wash the resulting growth from the agar surface with 50 milliliters of Medium 37 as described in § 436.102(b)(37).

[39 FR 18944, May 30, 1974, as amended at 40 FR 52004, Nov. 7, 1975; 42 FR 14092, Mar. 15, 1977; 42 FR 18058, Apr. 5, 1977; 44 FR 10378, Feb. 20, 1979; 47 FR 22514, May 25, 1982; 47 FR 27552, June 25, 1982]

§ 436.104 Penicillin activity.

Use penicillin-free equipment and glassware.

(a) *Preparation of inoculated plates.* Proceed as directed in § 436.105(a), using 10 milliliters of medium 1 for the base layer. For the seed layer, use 4 milliliters of medium 4, inoculated with the amount of test organism C which gave the clearest, sharpest zones of inhibition measuring 17 to 21 millimeters in diameter when standardized as described in § 436.103(b)(1)(ii). Use the plates the same day they are prepared.

(b) *Preparation of working standard stock solutions and standard response lines solutions.* Proceed as directed for penicillin G in § 436.105(b), except dilute the working standard stock solution to a final concentration of 100 units of penicillin G per milliliter and use the following final concentrations for the standard response line: 0.005, 0.0125, 0.025, 0.050, 0.100, and 0.200 unit of penicillin G per milliliter. The 0.050 unit of penicillin G–per–milliliter solution is the reference concentration of the assay.

(c) *Sample preparation.* Dissolve 1.0 gram of the sample in sufficient distilled water to make 18 milliliters. Filter if not clear. Transfer 9.0 milliliters to a separatory funnel, and add 20 milliliters of amyl acetate. Add 1 milliliter of 10 percent potassium phosphate buffer, pH 2.5 (solution 11 as described in § 436.101), shake, allow to separate, and draw off the aqueous layer into a second separatory funnel. Check the pH of the aqueous solution with pH paper, and readjust with concentrated hydrochloric acid if the pH is three or above. Extract again with 20 milliliters or amyl acetate, discard the aqueous phase, and combine the amyl acetate extracts. Wash the extracts with 10 milliliters of 1 percent potassium phosphate buffer, pH 2.5, and discard the

buffer wash. Extract the penicillin from the amyl acetate with a 10–milliliter aliquot of 1 percent potassium phosphate buffer, pH 6.0 (solution 1 as described in § 436.101). This is the assay solution.

(d) *Procedure for assay*. For the standard response line, use a total of 15 plates (three plates for each response line solution, except the reference concentration solution, which is included on each plate). On each set of three plates, fill three alternate cylinders with the reference concentration solution and the other three cylinders with the concentration of the response line under test. Thus, there will be 45 reference concentration zones of inhibition and nine zones of inhibition for each of the other concentrations of the response line. Treat a portion of the sample solution (2 to 5 milliliters) with 0.1 milliliter of penicillinase solution and incubate at 37° C. for 1 hour. For each sample tested, use three plates. On each plate fill two cylinders with the 0.050 unit of penicillin G per milliliter standard, two cylinders with the untreated sample, and two cylinders with the penicillinase-treated sample. Incubate all plates, including those of the standard response line, overnight at 30° C. A zone of inhibition with the untreated sample and no zone with the penicillinase-treated sample are a positive test for penicillin. If a positive test is obtained, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimeter rule, calipers, or an optical projector.

(e) *Estimation of penicillin G activity*. To prepare the standard response line, average the diameters of the standard reference concentration and average the diameters of the standard response line concentration tested for each set of three plates. Average also all 45 diameters of the reference concentration. The average of the 45 diameters of the reference concentration is the correction point of the response line. Correct the average diameter obtained for each concentration to the figure it would be if the average reference concentration diameter for that set of three plates were the same as the correction point. Thus, if in correcting the 0.025 penicillin G concentration, the average of the 45 readings of the 0.050 unit of penicillin G–per–milliliter concentration is 18.5 millimeters and the average of the 0.050 unit of pencillin G–per milliliter concentration of this set of three plates is 18.3 millimeters, the correction is +0.2 millimeters. If the average reading of the 0.025 unit of pencillin G–per–milliliter concentration of these same three plates is 15.5 millimeters, the corrected value is 15.7 millimeters. Plot these corrected values, including the average of the 0.050 unit of penicillin G-per-milliliter concentration, on semilogarithmic graph paper using the pencillin concentration in units per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the line of best fit through these points. To estimate the sample potency, average the zone diameters of the standard and the zone diameters of the sample on the three plates used. If the average zone diameter of the sample is lower than that of the standard, subtract the difference between them from the reference concentration diameter of the standard response line. From the response line, read the concentrations corresponding to these corrected values of zone diameters. Multiply the concentration by the dilution factor to obtain the units of penicillin G per sample size tested.

[39 FR 18944, May 30, 1974, as amended at 41 FR 34743, Apr. 17, 1976]

§ 436.105 Microbiological agar diffusion assay.

Using the sample solution prepared as described in the section for the particular antibiotic to be tested, proceed as described in paragraphs (a), (b), (c), and (d) of this section.

(a) *Preparation of inoculated plates.* For each antibiotic listed in the table in this paragraph, select the media (as listed by medium number in § 436.102(b)), the amount of media to be used in the base and seed layers, the test organism (as listed in § 436.103(a)), and the suggested inoculum and prepare the inoculated plates as follows: Prepare the base layer by adding the appropriate amount of melted agar to each Petri dish (nominal dimensions 20 by 100 millimeters). Distribute the agar

evenly in each dish on a flat, level surface, placing a cover on each plate in turn; if a nonporous cover is used, leave it slightly ajar to prevent accumulation of condensed moisture from the hot agar base layer. After the agar hardens, seat the nonporous cover on each plate. To prepare the seed layer, add the suggested inoculum of the test organism suspension to a sufficient amount of agar, which has been melted and cooled to 48° C–50° C. Swirl the flask to obtain a homogeneous suspension, and add the appropriate amount of the inoculated media to each of the plates containing the uninoculated base agar. Spread evenly over the agar surface, cover, and allow to harden on a flat, level surface. After the agar has hardened, place 6 cylinders described in § 436.100(a)(1) on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8—centimeter radius.

Antibiotic	Media to be used (as listed by medium number in § 436.102(b))		Milliliters of media to be used in the base and seed layers		Test organism	Suggested volume of standardized inoculum to be added to each 100 milliliters of seed agar	Incubation Temperature for the plates
	Base layer	Seed layer	Base layer	Seed layer			
						Milliliters	*Degrees C.*
Amoxicillin	11	11	21	4	C	0.5	32–35
Amphotericin B	None	19	None	8	E	1.0	29–31
Ampicillin	11	11	21	4	C	0.5	32–35
Bacitracin	2	1	21	4	B	0.3	32–35
Bacitracin	2	1	21	4	L	0.3	32–35
Bleomycin	35	35	10	6	X	1.0	32–35
Carbenicillin	9	10	21	4	W	[2] 0.5	36–37.5
Cefactor	2	1	21	5	A	0.05	36–37.5
Cefadroxil	2	1	21	4	A	0.05	36–37.5
Cefamandole	2	1	21	5	A	0.06	36–37.5
Cefazolin	2	1	21	4	A	0.05	32–35
Cefotaxime	2	1	21	5	A	0.1	36–37.5
Cefoxitin	2	1	21	5	A	0.1	36–37.5
Cephalexin	2	1	21	4	A	0.05	32–35
Cephaloglycin	2	1	21	4	A	0.2	32–35
Cephaloridine	2	1	21	4	A	0.1	32–35
Cephalothin	2	1	21	4	A	0.1	32–35
Cephapirin	2	1	21	4	A	0.08	32–35
Cephradine	2	1	21	4	A	0.05	32–35
Clindamycin	11	11	21	4	C	1.5	36–37.5
Cloxacillin	2	1	21	4	A	0.1	32–35
Colistimethate, sodium	9	10	21	4	F	0.1	36–37.5
Colistin	9	10	21	4	F	0.1	36–37.5
Cyclacillin	11	11	21	4	C	0.5	36–37.5
Dactinomycin	5	5	10	4	H	([1])	36–37.5
Dicloxacillin	2	1	21	4	A	0.1	32–35
Dihydrostreptomycin	5	5	21	4	H	([1])	36–37.5
Erythromycin	11	11	21	4	C	1.5	32–35
Gentamicin	11	11	21	4	D	0.03	36–37.5
Kanamycin B	5	5	21	4	H	([1])	36–37.5
Methicillin	2	1	21	4	A	0.3	32–35
Mitomycin	8	8	10	4	H	0.5	36–37.5
Nafcillin	2	1	21	4	A	0.3	32–35
Natamycin	None	19	None	8	E	0.8	29–31
Neomycin	11	11	21	4	A	0.4	32–35
Neomycin	11	11	21	4	D	1.0	36–37.5
Netilmicin	11	11	20	5	D	0.25	36–37.5
Novobiocin	2	1	21	4	D	4.0	34–36
Nystatin	None	19	None	8	T	1.0	29–31
Oleandomycin	11	11	21	4	D	1.0	36–37.5
Oxacillin	2	1	21	4	A	0.3	32–35
Paromomycin	11	11	21	4	D	2.0	36–37.5
Penicillin G	2	1	21	4	A	1.0	32–35
Penicillin V	2	1	21	4	A	1.0	32–35
Plicamycin	8	8	10	4	A	0.1	32–35
Polymyxin B	9	10	21	4	F	0.1	36–37.5
Rifampin	2	2	21	4	H	0.1	29–31
Sisomicin	11	11	21	4	D	0.03	36–37.5
Streptomycin	5	5	21	4	H	([1])	36–37.5
Ticarcillin	38	38	21	4	Y	1.5	36–37.5

Antibiotic	Media to be used (as listed by medium number in § 436.102(b))		Milliliters of media to be used in the base and seed layers		Test organism	Suggested volume of standardized inoculum to be added to each 100 milliliters of seed agar	Incubation Temperature for the plates
	Base layer	Seed layer	Base layer	Seed layer			
						Milliliters	*Degrees C.*
Vancomycin	8	8	10	4	H	(1)	36–37.5

1 Determine the amount of the inoculum by the use of test plates.
2 Use dilution of the suspension that gives 25 percent light transmission in lieu of the stock suspension.

(b) *Preparation of working standard stock solutions and standard response line solutions.* For each antibiotic listed in the table in this paragraph, select the working standard drying conditions, solvent(s), concentrations, and storage time for the standard solutions and proceed as follows: If necessary, dry the working standard as described in § 436.200; dissolve and dilute an accurately weighed portion to the proper concentration to prepare the working standard stock solution. Store the working standard stock solution under refrigeration and do not use longer than the recommended storage time. Further dilute an aliquot of the working standard stock solution to the proper concentrations to prepare the standard response line solutions. The reference concentration of the assay is the mid concentration of the response line.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in § 436.200)	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration units or milligrams per milliliter	Storage time under refrigeration	Diluent	Final concentrations, units or micrograms of antibiotic activity per milliliter
Amoxicillin	Not dried		Distilled water	1.0 mg	7 days	3	0.064, 0.080, 0.100, 0.125 and 0.156, µg. (Prepare the standard response line simultaneously with the sample solution.)
Amphotericin B	1		Dimethylsulfoxide	1 mg.[1]	Use same day	10	0.64 0.80, 1.00, 1.25, 1.56 µg. (Prepare the standard response line simultaneously with the sample solution.)
Ampicillin	Not dried		Distilled water	0.1	1 week	3	0.064, 0.080, 0.100, 0.125 0.156 µg. (Prepare the standard response line simultaneously with the sample solution.)
Bacitracin zinc	1		0.01*N* HCl	100 units	Use same day	1	0.64, 0.80, 1.0, 1.25, 1.56 units.
Bleomycin	7		16	2 units	2 weeks	16	0.01, 0.02, 0.04, 0.08, 0.16 unit.
Carbenicillin	Not dried		1	1 mg	2 weeks	1	12.8, 16.0, 20.0, 25.0, 31.2 µg.
Cefaclor	Not dried		1	1 mg	1 day	1	3.2, 4.0, 5.0, 6.25, 7.81 µg.
Cefadroxil	Not dried		1	1mg	Use same day	1	12.8,16.0, 20.0, 25.0, and 31.2µg.
Cefamandole	Not dried		3	1mg[5]	1 day	1	1.28, 1.60, 2.00, 2.50, 3.12 µg.
Cefazolin	Not dried	10,000 µg per ml. in solution 6.	1	1 mg	5 days	1	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Cefotaxime	Not dried	1		1 mg	Use same day	1	6.4, 8.0, 10.0, 12.5, 15.6 µg.
Cefoxitin	Not dried		1	1 mg	Use same day	1	12.8, 16.0, 20.0, 25, 31.2 µg.
Cephalexin	Not dried		1	1 mg	7 days	1	12.8, 16.0, 20.0, 25.0, 31.2 µg.
Cephaloglycin	Not dried		Distilled water	100 µg	1 week	4	6.4, 8.0, 10.0, 12.5, 15.6 µg.
Cephaloridine	1		1	1 mg	5 days	1	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Cephalothin	1		1	1 mg	5 days	1	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Cephapirin	Not dried		1	1 mg	3 days	1	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Cephradine	Not dried		1	1 mg	5 days	1	6.4, 8.0, 10.0, 12.5, 15.6 µg.
Clindamycin	Not dried		Distilled water	1 mg	1 month	3	0.64, 0.80, 1.00, 1.25 1.56 µg.
Cloxacillin	Not dried		1	1 mg	7 days	1	3.20, 4.00, 5.00, 6.25, 7.81 µg.
Colistimethate, sodium	1	10,000 µg. per ml. in distilled water.	6	1 mg	Use same day	6	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Colistin	1	10,000 µg. per ml. in distilled water.	6	1 mg	2 weeks	6	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Cyclacillin	Not dried		Distilled water	1 mg	1 day	3	0.64, 0.80, 1.0, 1.25, 1.56 µg. (Prepare the standard response line simultaneously with the sample solution.)
Dactinomycin	1	10,000 µg. per ml. in methyl alcohol.	3	1 mg	3 months	3	0.50, 0.71, 1.00, 1.41, 2.00 µg.
Dicloxacillin	Not dried		1	1 mg	7 days	1	3.20, 4.00, 5.00, 6.25, 7.81 µg.
Dihydrostreptomycin	5		3	1 mg	30 days	3	0.64, 0.80, 1.00, 1.25, 1.56 µg.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in § 436.200)	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration units or milligrams per milliliter	Storage time under refrigeration	Diluent	Final concentrations, units or micrograms of antibiotic activity per milliliter
Erythromycin	1	10,000 µg. per ml. in methyl alcohol.	3	1 mg	14 days	3	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Gentamicin	3		3	1 mg	1 month	3	0.064, 0.080, 0.100, 0.125, 0.156 µg.
Kanamycin B (use the kanamycin sulfate working standard).	Not dried		3	1 mg	1 month	3	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Methicillin	Not dried		1	1 mg	4 days	1	6.4, 8.0, 10.0, 12.5, 15.6 µg.
Mitomycin	Not dried		1	1 mg	14 days	1	0.50, 0.71, 1.0, 1.41, 2.0 µg.
Nafcillin	Not dried		1	1 mg	2 days	1	1.28, 1.60, 2.00, 2.50, 3.12 µg.
Natamycin	Not dried		Dimethylsulfoxide	1 mg [7]	Use same day	10	3.20, 4.00, 5.00, 6.25, 7.81 µg. (Prepare the standard response line solutions simultaneously with the sample solution to be tested using red low actinic glassware. Use solutions within 2 hours after preparation.)
Neomycin	1		3	1 mg	2 weeks	3	0.64, 0.80, 1.00, 1.25, 1.56 µg. (if test organism D is used); 6.4, 8.0, 10.0, 12.5, 15.6 µg. (if test organism A is used).
Netilmicin	Not dried [9]		3	1 mg	7 days	3	0.064, 0.080, 0.100, 0.125, 0.156 µ g.
Novobiocin	5	10,000 µg. per ml. in absolute ethyl alcohol.	3	1 mg	5 days	6	0.320, 0.400, 0.500, 0.625, 0.781 µg.
Nystatin [10]	4		Dimethylformamide	1,000 units [2]	Use same day	6	12.8, 16.0, 20.0, 25.0, 31.2 units. (Prepare the standard response line solutions simultaneously with the sample solution to be tested using red low actinic glassware.)
Oleandomycin	Not dried	10,000 µg. per ml. in ethyl alcohol.	3	1 mg	30 days	3	3.20, 4.00, 5.00, 6.25, 7.81 µg.
Oxacillin	Not dried		1	1 mg	3 days	1	3.20, 4.00, 5.00, 6.25, 7.81 µg.
Paromomycin	1		3	1 mg	3 weeks	3	0.64, 0.80, 1.00, 1.25, 1.56 µg.
Penicillin G	Not dried		1	1,000 units	4 days	1	0.64, 0.80, 1.00, 1.25, 1.56 units.
Penicillin V Potassium	Not dried		1	100 units	4 days	1	0.64, 0.80, 1.00, 1.25, 1.56 units.
Plicamycin	7		Distilled water	0.1 mg	1 day	1	0.50, 0.7, 1.00, 1.41, 2.00 µg.
Polymyxin B	1	Distilled water [3]	6	10,000 units	2 weeks	6	6.4, 8.0, 10.0, 12.5, 15.6 units.
Rifampin	Not dried		Methyl alcohol	1 mg	1 day	1	3.20, 4.00, 5.00, 6.25, 7.81, µg.
Streptomycin	1		3	1 mg	30 days	3	0.64, 0.80, 1.00, 1.25, 1.56 µg.

Sisomicin[6]	Not dried[8]		3	1 mg	14 days	3	0.064, 0.080, 0.100, 0.125, 0.156 µg.
Ticarcillin	Not dried		1	1 mg	1 day	1	3.20, 4.00, 5.00, 6.25, 7.81 µg.
Vancomycin	1		Distilled water	1 mg	1 week	4	6.4, 8.0, 10.0, 12.5, 15.6 µg.

[1] Further dilute aliquots of the working standard stock solution with dimethylsulfoxide to give concentrations of 12.8, 16, 20.0, 25, and 31.2 micrograms per milliliter.
[2] Further dilute aliquots of the working standard stock solution with dimethylformamide to give concentrations of 256, 320, 400, 500, and 624 units per milliliter.
[3] Add 2 milliliters of distilled water for each 5 milligrams of weighed working standard material.
[4] Further dilute aliquots of the working standard stock solution with dimethylformamide to give concentrations of 64, 80, 100, 125, and 156 micrograms per milliliter.
[5] The final concentration of the working standard stock solution is allowed to hydrolyze in a 37° C. constant temperature water bath for 60 minutes.
[6] Working standard should be stored below minus 20° C under an atmosphere of nitrogen. Sisomicin is hygroscopic and care should be exercised during weighing.
[7] Further dilute aliquots of the working standard stock solution with dimethylsulfoxide to give concentrations 64.0, 80.0, 100, 125, and 156 micrograms per milliliter.
[8] Weigh a separate portion of the working standard and determine the loss on drying by the method described in § 436.200(c) of this chapter. Use this value to determine the anhydrous content of the working standard.
[9] Working standard should be stored below minus 10° C under an atmosphere of nitrogen. Netilmicin sulfate is hygroscopic and care should be exercised during weighing.
[10] For assay of nystatin pastilles, use 80 percent aqueous dimethylformamide as the initial solvent and as diluent for all dilutions where dimethylformamide is required.

(c) *Procedure for assay*. For the standard response line, use a total of 12 plates—three plates for each response line solution, except the reference concentration solution which is included on each plate. On each set of three plates, fill three alternate cylinders with the reference concentration solution and the other three cylinders with the concentration of the response line under test. Thus, there will be 36 reference concentration zones of inhibition and nine zones of inhibition for each of the four other concentrations of the response line. For each sample tested use three plates. Fill three alternate cylinders on each plate with the standard reference concentration solution and the other three cylinders with the sample reference concentration solution. After all the plates have incubated for 16 to 18 hours at the appropriate incubation temperature for each antibiotic listed in the table in paragraph (b) of this section, measure the diameters of the zones of inhibition using an appropriate measuring device such as a millimeter rule, calipers, or an optical projector.

(d) *Estimation of potency*. To prepare the standard response line, average the diameters of the standard reference concentration and average the diameters of the standard response line concentration tested for each set of three plates. Average also all 36 diameters of the reference concentration for all four sets of plates. The average of the 36 diameters of the reference concentration is the correction point of the response line. Correct the average diameter obtained for each concentration to the figure it would be if the average reference concentration diameter for that set of three plates were the same as the correction point. Thus, if in correcting the highest concentration of the response line, the average of the 36 diameters of the reference concentration is 16.5 millimeters and the average of the reference concentration of the set of three plates (the set containing the highest concentration of the response line) is 16.3 millimeters, the correction is +0.2 millimeter. If the average reading of the highest concentration of the response line of these same three plates is 16.9 millimeters, the corrected diameter is then 17.1 millimeters. Plot these corrected diameters, including the average of the 36 diameters of the reference concentration on 2-cycle semilog paper, using the concentration of the antibiotic in micrograms or units per milliliter as the ordinate (the logarithmic scale), and the diameter of the zone of inhibition as the abscissa. The response line is drawn either through these points by inspection or through points plotted for highest and lowest zone diameters obtained by means of the following equation:

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

where:

L=Calculated zone diameter for the lowest concentration of the standard response line;

H=Calculated zone diameter for the highest concentration of the standard response line;

c=Average zone diameter of 36 readings of the reference point standard solution;

a, b, d, e=Corrected average values for the other standard solutions, lowest to highest concentration, respectively.

To estimate the potency of the sample, average the zone diameters of the standard and the zone diameters of the sample on the three plates used. If the average zone diameter of the sample is larger than that of the standard, add the difference between them to the reference concentration diameter of the standard response line. If the average zone diameter of the sample is lower than that of the standard, subtract the difference between them from the reference concentration diameter of the standard response line. From the response line, read the concentrations corresponding to these corrected values of zone diameters. Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

[39 FR 18944, May 30, 1974]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 436.105, see the List of CFR Sections Affected appearing in the Finding Aids section of this volume.

§ 436.106 Microbiological turbidimetric assay.

Using the sample solution prepared as described in the section for the particular antibiotic to be tested, proceed as described in paragraphs (a), (b), and (c) of this section.

(a) *Preparation of working standard stock solutions and standard response line solutions.* For each antibiotic listed in the table in this paragraph, select the working standard, drying conditions, solvent(s), concentrations, and storage time for the standard solutions and proceed as follows: If necessary, dry the working standard as described in § 436.200; dissolve and dilute an accurately weighed portion to the proper concentration for the working standard stock solution. Store the working standard stock solution under refrigeration and do not use longer than the recommended storage time. Prepare the proper concentrations for the standard response line solutions by further diluting an aliquot of the working standard stock solution. The reference concentration of the assay is the mid concentration of the standard response line.

Antibiotic	Working standard stock solutions					Standard response line concentrations	
	Drying conditions (method number as listed in § 436.200)	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration units or milligrams per milliliter	Storage time under refrigeration	Diluent (solution number as listed in § 436.101(a))	Final concentrations—units or micrograms of antibiotic activity per milliliter
Amikacin	Not dried		Distilled water	1 mg	2 weeks	Distilled water	8.0, 8.9, 10.0, 11.2, 12.5 µg.
Candicidin [1]	6		Dimethyl sulfoxide	1 mg	Use same day	Distilled water	0.030, 0.043, 0.060, 0.085, 0.120 µg. (Prepare standard response line simultaneously with the sample solution.)
Capreomycin	5	Distilled water		1 mg	7 days	Distilled water	80.0, 89.0, 100.0, 112,0, 125.0 µg.
Chloramphenicol	Not dried	Ethyl alcohol (10,000 µg. per ml.).	Distilled water	1 mg	1 month	Distilled water	2.00, 2.24, 2.50, 2.80, 3.12 µg.
Chlortetracycline	Not dried		0.01*N* HCl	1 mg	4 days	Distilled water	0.048, 0.054, 0.060, 0.067, 0.075 µg.
Cycloserine	1		Distilled water	1 mg	1 month	Distilled water	40.0, 44.5, 50.0, 56.0, 62.5 µg.
Demeclocycline	1		0.1*N* HCl	1 mg	4 days	Distilled water	0.080, 0.089, 0.100, 0.112, 0.125 µg.
Dihydrostreptomycin	5		Distilled water	1 mg	30 days	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 µg.
Doxycycline	Not dried		0.1*N* HCl	1 mg	5 days	Distilled water	0.080, 0.089, 0.100, 0.112, 0.125 µg.
Gramicidin	1		alcohol U.S.P. XX	1 mg	30 days	alcohol U.S.P. XX.	0.032, 0.0356, 0.040, 0.0448, 0.050 µg.
Kanamycin	Not dried		Distilled water	1 mg	1 month	Distilled water	8.0, 8.9, 10.0, 11.2, 12.5 µg.
Lincomycin	Not dried		Distilled water	1 mg	1 month	Distilled water	0.400, 0.447, 0.500, 0.559, 0.625 µg.
Meclocycline	Not dried		13	1 mg	Use same day	Distilled water	0.048, 0.054, 0.06, 0.067, 0.075 µg.
Methacycline	1		Distilled water	1 mg	7 days	Distilled water	0.048, 0.054, 0.060, 0.067, 0.075 µg.
Oxytetracycline	Not dried		0.1*N* HCl	1 mg	4 days	Distilled water	0.192, 0.215, 0.240, 0.268, 0.300 µg.
Rolitetracycline	1		Distilled water	1 mg	1 day	Distilled water	0.192, 0.215, 0.240, 0.268, 0.300 µg.
Spectinomycin	Not dried		Distilled water	1 mg	1 month	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 µg.
Streptomycin	1		Distilled water	1 mg	30 days	Distilled water	24.0, 26.8, 30.0, 33.5, 37.5 µg.
Tetracycline	Not dried		0.1*N* HCl	1 mg	1 day	Distilled water	0.192, 0.215, 0.240, 0.268, 0.300 µg.
Tobramycin	Not dried		Distilled water	1 mg	2 weeks	Distilled water	2.00, 2.236, 2.5, 2.795, 3.125 µg.
Troleandomycin	1		15	1 mg	Use same day	Distilled water	20.0, 22.25, 25.0, 28.0, 31.25 µg.
Tyrothricin [2]							

[1] Use sterile equipment for all stages of this assay.
[2] The gramicidin working standard and the gramicidin standard response line concentrations are used for the assay of tyrothricin.

(b) *Procedure for assay.* For each antibiotic listed in the table in this paragraph, select the test organism (as listed in § 436.103(a)), nutrient broth (as listed by medium number in § 436.102(b)), and suggested inoculum and proceed as follows: Place 1.0 milliliter (or 0.1 milliliter in the case of gramicidin and tyrothricin) of each concentration of the standard response line (prepare as described in paragraph (a) of this section) and of the sample solution in each set of three replicate tubes (as described in § 436.100(b)(1)). Fifteen tubes are used for the five-point standard response line and three for each sample. To each tube add 9 milliliters of the inoculated broth and place immediately in a water bath at the appropriate temperature for 2 to 4 hours. The exact length of the incubation period should be determined by observation of growth in the reference concentration tube of the standard. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable photoelectric colorimeter, at a wavelength of 530 millimicrons. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of nutrient broth and formaldehyde used in the assay.

NOTE: The amount of working standard and sample solutions may be reduced as long as all other solutions used are reduced proportionately.

Antibiotic	Test organism	Medium (nutrient broth)	Suggested volume of standardized inoculum to be added to each 100 milliliters of medium (nutrient broth)	Incubation temperature
Amikacin	A	3	0.1	36–37.5
Candicidin [1]	E	13	0.2	27–29
Capreomycin	I	3	0.05	36–37.5
Chloramphenicol	J	3	0.7	36–37.5
Chlortetracycline	A	3	0.1	36–37.5
Cycloserine	A	3	0.4	36–37.5
Demeclocycline	A	3	0.1	36–37.5
Dihydrostreptomycin	I	3	0.1	36–37.5
Doxycycline	A	3	0.1	36–37.5
Gramicidin	K	3	1.0	36–37.5
Kanamycin	A	3	0.2	36–37.5
Lincomycin	A	3	0.1	36–37.5
Meclocycline	A	3	0.2	36–37.5
Methacycline	A	3	0.1	36–37.5
Oxytetracycline	A	3	0.1	36–37.5
Rolitetracycline	A	3	0.1	36–37.5
Spectinomycin	J	3	0.1	36–37.5
Streptomycin	I	3	0.1	36–37.5
Tetracycline	A	3	0.1	36–37.5
Tobramycin	A	3	0.15	36–37.5
Troleandomycin	I	3	0.1	36–37.5
Tyrothricin	K	3	1.0	36–37.5

[1] Use sterile equipment for all stages of this assay.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

where:

L=Calculated absorbance value for the lowest concentration of the standard response line.

H=Calculated absorbance value for the highest concentration of the standard response line.

a, b, c, d, e=Average absorbance values for each concentration of the standard response line, lowest to the highest, respectively.

(c) *Estimation of potency.* To prepare the standard response line, plot the average absorbance values for each concentration of the standard response line on one-cycle semilogarithmic graph paper with the absorbance values on the arithmetric scale and concentrations on the logarithmic scale. The response line is drawn either through these points by inspection or through points plotted for highest and lowest absorbance values obtained by means of the following equations.

To estimate the potency of the sample, average the absorbance values for the sample and determine the antibiotic concentration from the standard response line. Multiply the concentration by the appropriate dilution factor to obtain the antibiotic content of the sample.

[39 FR 18944, May 30, 1974, as amended at 40 FR 57797, Dec. 12, 1975; 41 FR 49483, Nov. 9, 1976; 44 FR 22057, Apr. 13, 1979; 46 FR 3835, 3839, Jan. 16, 1981; 46 FR 16682, Mar. 13, 1981; 46 FR 33512, June 30, 1981; 46 FR 61072, Dec. 15, 1981; 47 FR 22515, May 25, 1982; 47 FR 23708, June 1, 1982; 48 FR 3960, Jan. 28, 1983; 53 FR 32607, Aug. 26, 1988]

Subpart E—General Chemical Tests for Antibiotics

§ 436.200 Loss on drying.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* In an atmosphere of about 10 percent relative humidity, grind the sample, if necessary, to obtain a fine powder. When tablets, troches, or capsules are to be tested, use four tablets, troches, or capsules in preparing the sample. Transfer about 100 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent, such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent of loss.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use a tared weighing bottle or weighing tube equipped with a capillary-tube stopper, the capillary having an inside diameter of 0.20–0.25 millimeter, and place it in a vacuum oven without removing the stopper.

(c) *Method 3.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 110° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(d) *Method 4.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 2 hours.

(e) *Method 5.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 100° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(f) *Method 6.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 40° C. and a pressure of 5 millimeters of mercury or less for 3 hours.

(g) *Method 7.* Proceed as directed in paragraph (a) of this section, except dry the sample at a temperature of 25° C. and a pressure of 5 millimeters of mercury or less for 4 hours.

(h) *Method 8.* Proceed as directed in paragraph (a) of this section, except transfer approximately 300 milligrams of the sample to a tared weighing bottle equipped with a ground-glass stopper; dry the sample at a temperature of 25 ° C and a pressure of 5 millimeters of mercury or less for 4 hours, and then dry the sample at a temperature of 100 °C and a pressure of 5 millimeters of mercury or less for 3 additional hours.

(i) *Method 9.* Use a suitable thermogravimetric apparatus prepared for vacuum operation. Rapidly and thoroughly grind a portion of the sample and promptly transfer 5 to 10 milligrams to the sample pan. Place the system under vacuum and allow it to come to equilibrium before heating. Obtain an accurate sample weight and

continuously record the weight loss as the sample is heated at a rate of 20° per minute from room temperature to about 200 ° C. The weight loss plateau, or inflection, at about 150 ° C is taken as the total volatile weight loss. Calculate the percent weight loss on drying.

[39 FR 18944, May 30, 1974, as amended at 50 FR 48397, Nov. 25, 1985; 51 FR 11572, Apr. 4, 1986]

§ 436.201 Moisture determination.

(a) *Equipment*—(1) *Apparatus.* Use a closed system consisting of all glass automatic burettes, platinum electrodes, and a magnetic stirrer connected to a suitable electrometric apparatus. This apparatus embodies a simple electrical circuit which serves to pass 5 to 10 microamperes of direct current between a pair of platinum electrodes immersed in the solution to be titrated. At the endpoint of the titration a slight excess of the reagent increases the flow of current to between 50 and 150 microamperes for 30 seconds or longer, depending upon the solution being titrated.

(2) *Titrating vessel.* Use a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator.

(b) *Reagents*—(1) *Karl Fischer reagent.* Dissolve 125 grams of iodine in 170 milliliters of pyridine, add 670 milliliters of methanol and cool. To 100 milliliters of pyridine kept in an ice bath, add sulfur dioxide until the volume reaches 200 milliliters. Slowly add this solution to the cooled iodine-methanol-pyridine mixture and shake well. (A commercially prepared Karl Fischer reagent, pyridine containing or pyridine-free, may be used.) Preserve the reagent in glass-stoppered bottles protected from light and from moisture in the air.

(2) *Methanol solution.* Add sufficient water (usually 2 milligrams per milliliter) to methanol so that each milliliter of the resulting methanol solution is equivalent to about 0.5 milliliter of Karl Fischer reagent.

(3) *Solvents*—(i) *Solvent A.* Methanol:chloroform:carbon tetrachloride (1:2:2 by volume).

(ii) *Solvent B.* Chloroform:carbon tetrachloride (1:1 by volume).

(iii) *Solvent C.* Anhydrous methanol.

(c) *Standardization of reagents*—(1) *Water equivalence of Karl Fischer reagent.* Standardize the Karl Fischer reagent no longer than 1 hour before use by one of the following methods.

(i) Accurately weigh 25–35 milligrams of water into a dry titration vessel and add 20 milliliters of solvent A. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e = \frac{W}{V_T - V_A}$$

where:

e=Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W=Milligrams of water;

V_T=Milliliters of Karl Fischer reagent used;

V_A=Milliliters of Karl Fischer reagent equivalent to the 20 milliliters of solvent A, determined as directed in paragraph (c)(3) of this section.

(ii) Accurately weigh about 25–35 milligrams of water into a dry titration vessel, add an excess of Karl Fischer reagent, start the stirrer, and titrate to the endpoint with methanol solution. Calculate the water equivalence of the Karl Fischer reagent as follows:

$$e = \frac{W}{V_T - V_A}$$

where:

e=Water equivalence of the Karl Fischer reagent in terms of milligrams of water per milliliter;

W=Milligrams of water;

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used;

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c)(2) of this section.

(2) *Karl Fischer reagent equivalence of methanol solution.* Titrate a known volume of Karl Fischer reagent with methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution as follows:

$$e = \frac{W}{(V_T - V_m) \times f}$$

where:

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution;

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used.

(3) *Karl Fischer reagent equivalence of solvents.* (i) Solvent A: Use 20 milliliters of solvent A as the sample. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent.

(ii) Solvent B: Use 10 milliliters of solvent B as the sample. Add an excess of Karl Fischer reagent to the sample and start the stirrer. Titrate to the endpoint with methanol solution.

(iii) *Solvent C.* Use 20 milliliters of solvent C as the sample. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent.

(iv) Calculate the Karl Fischer reagent equivalence of the solvents as follows:

$V_A = V_C = V_T$,

$V_B = (V_T - V_m)$ X f

where:

V_A, V_B, and V_C=Milliliters of Karl Fischer reagent equivalent to the aliquots used of solvents A, B, and C, respectively;

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used;

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c)(2) of this section.

(d) *Sample preparation*—(1) *Powders.* In the case of tablets, grind 4 tablets to a fine powder. In the case of capsules containing enteric-coated pellets, grind the pellets to a fine power. If the maximum moisture limit is greater than 1 percent, accurately weigh about 300 milligrams of the sample into a dry titrating vessel. If the maximum moisture limit is less than 1 percent, accurately weigh 1 to 2 grams of the sample. Proceed as directed in paragraph (e)(1) or (2) of this section.

(2) *Ointments and oils.* (i) Transfer about 1 to 2 grams, accurately weighed, into a dry titrating vessel. Proceed as directed in paragraph (e)(1) of this section; or

(ii) Transfer about 1 to 2 grams, accurately weighed, into a dry titrating vessel. Add 10 milliliters of solvent B and proceed as directed in paragraph (e)(2) of this section.

(3) *Aerosols with propellant.* Place the immediate container to be tested in a suitable freezing unit having a temperature of not higher than 0° C. for at least 2 hours. Remove the container from the freezing unit, puncture it, mix the entire contents by swirling. Proceed as directed in paragraph (e)(3) of this section, using an accurately measured 10-milliliter aliquot from the container as the sample and allowing the solution to warm to at least 10° C. before determining the endpoint.

(4) *Hygroscopic powders.* Weigh the immediate container. Using a suitable dry hypodermic needle and syringe, inject 3 milliliters of anhydrous methanol into the container and shake to dissolve the contents. Using the same syringe, remove the withdrawable contents and transfer into the titration vessel. Rinse the syringe and needle by drawing in an additional 3 milliliters of anhydrous methanol. Add the rinsings to the titration vessel. Titrate the solution immediately, proceeding as directed in paragraph (e)(3) of this section. Determine the Karl Fischer equivalent (in milliliters), if any, of the anhydrous methanol by titrating a blank of the same total volume used in preparing the sample and rinsing the syringe and needle. Dry the immediate container and its closure for three hours at 100° C., cool to room temperature in a desiccator, and weigh. Determine the weight of sample tested by subtracting the weight obtained from the original weight of the immediate container.

(5) *Solutions.* Proceed as directed in paragraph (e)(3) of this section, using about 1 to 2 grams of the sample, accurately weighted.

(e) *Titration procedures and calculations*—(1) *Procedure 1.* Add 20 milliliters of solvent A to the sample. Start the stirrer and titrate to the endpoint by adding measured quantities of Karl Fischer reagent. Determine the percent moisture in the sample as follows:

$$\text{Percent moisture} = \frac{(V_T - V_A) \times e \times 100}{W_s}$$

where:

e=Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c)(1) of this section;

V_T=Milliliters of Karl Fischer reagent used;

V_A=Milliliters of Karl Fischer reagent equivalent to the 20 milliliters of solvent A, determined as directed in paragarph (c)(3) of this section;

W_s=Weight of the sample in milligrams.

(2) *Procedure 2.* Add an excess of Karl Fischer reagent to the sample, start the stirrer, and titrate to the endpoint with methanol solution. Calculate the percent moisture in the sample as follows:

(i) For powders:

$$\text{Percent moisture} = \frac{(V_T - V_m f) \times e \times 100}{W_s}$$

(ii) For oils and ointments:

$$\text{Percent moisture} = \frac{(V_T - V_m f - V_B) \times e \times 100}{W_s}$$

where:

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used;

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c)(2) of this section.

V_B=Milliliters of Karl Fischer reagent equivalent to the 10 milliliters of solvent B determined as directed in paragraph (c)(3) of this section;

e=Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c)(1) of this section;

W_s=Weight of the sample in milligrams.

(3) *Procedure 3.* Add about 20 milliliters of solvent A to a dry titrating vessel and proceed as directed in titration procedure 1 or 2. Disregard the volume of reagents used to determine the endpoint. Promptly introduce an accurately weighed or measured quantity of sample into the titrating vessel and titrate to the endpoint using either titration procedure 1 or 2 without additional solvents. Calculate the percent moisture in the sample as follows:

(i) If titration procedure 1 is used:

$$\text{Percent moisture in weighed samples} = \frac{V_T \times e \times 100}{W_s}$$

$$\text{Percent moisture in aerosols} = \frac{V_T \times e}{\text{Milliliters of sample} \times 10}$$

Percent moisture in hygroscopic powders=

$$\text{Percent moisture in hygroscopic powders} = \frac{(V_T - V_b) \times e \times 100}{W_s}$$

(ii) If titration procedure 2 is used:

$$\text{Percent moisture in weighed samples} = \frac{(V_T - V_m f) \times e \times 100}{W_s}$$

$$\text{Percent moisture in aerosols} = \frac{(V_T - V_m f) \times e}{\text{Milliliters of sample} \times 10}$$

$$\text{Percent moisture in hygroscopic powders} = \frac{(V_T - V_m f - V_b) \times e \times 100}{W_s}$$

where:

V_T=Milliliters of Karl Fischer reagent used;

V_m=Milliliters of methanol solution used;

f=Milliliters of Karl Fischer reagent equivalent to each milliliter of methanol solution determined as directed in paragraph (c)(2) of this section;

V_b=Karl Fischer equivalent (in milliliters) of the methanol used as a sample solvent;

e=Water equivalence of the Karl Fischer reagent determined as directed in paragraph (c)(1) of this section.

[39 FR 18944, May 30, 1974, as amended at 48 FR 51292, Nov. 8, 1983; 50 FR 41679, Oct. 15, 1985; 51 FR 22071, June 18, 1986; 51 FR 27532, Aug. 1, 1986]

§ 436.202 pH.

(a) *Apparatus.* A suitable potentiometer fitted with two electrodes, one being constructed of glass and sensitive to hydrogen ion activity and the other being a calomel or a silver/silver chloride reference electrode. A combination electrode with glass electrode and reference electrode contained in the same system may be used.

(b) *Standardization.* Select two standard buffer solutions such that the expected pH value of the sample is within their pH range and is also within 2 pH

unit of one of the standard buffer solutions. Standardize the pH meter with the two buffer solutions. Make any necessary adjustment of the meter if the observed pH value of either standard solution differs by more than 0.05 pH units of its known value.

(c) *Sample preparation.* If necessary, dilute the sample with carbon dioxide-free distilled water to the concentration specified in the individual section for each antibiotic.

(d) *Test procedure.* Determine the pH of the sample at 25°±2° C. Rinse the electrode(s) between determinations first with distilled water and then with a portion of the next sample to be tested. Store electrode(s) with tips immersed in water.

[39 FR 18944, May 30, 1974, as amended at 42 FR 29857, June 10, 1977; 42 FR 31449, June 21, 1977]

§ 436.203 Crystallinity.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* To prepare the sample for examination, mount a few particles in mineral oil on a clean glass slide. Examine the sample by means of a polarizing microscope. The particles reveal the phenomena of birefringence and extinction positions on revolving the microscope stage.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except to prepare the sample for examination, mount a few particles in mineral oil, add 1 drop of ethyl alcohol, and allow to react for about 30 seconds.

§ 436.204 Iodometric assay.

(a) *Reagents.* (1) 0.01*N* Sodium thiosulfate (2.482 grams $Na_2S_2O_35H_2O$ and 125 milligrams Na_2CO_3 per liter).

(2) 1.0*N* Sodium hydroxide.

(3) 1.2*N* Hydrochloric acid.

(4) 0.01*N* Iodine solution (prepared from 0.1*N* iodine U.S.P.).

(5) Starch iodide paste, T.S. (U.S.P.).

(b) *Preparation of sample and working standard solutions*—(1) *Workingstandard solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of standard solution
Amoxicillin	None	Distilled water	1.0 mg.
Ampicillin	do	do	1.25 mg.
Cephalexin	do	do	2 mg.
Cephaloridine	do	do	2 mg.
Cephalothin	do	do	2 mg.
Cephapirin	do	do	2 mg.
Cloxacillin	do	do	1.25 mg.
Cyclacillin	do	do	1.0 mg.
Dicloxacillin	do	1	1.25 mg.
Methicillin	do	1	1.25 mg.
Nafcillin	do	1	1.25 mg.
Oxacillin	do	1	1.25 mg.
Penicillin G	do	1	2,000 units.
Penicillin V potassium	do	1	2,000 units.

(2) *Bulk antibiotic solutions.* From the following table, select the initial solvent, diluent, and final concentration as listed for each antibiotic. Dissolve an accurately weighed aliquot (approximately 30 to 60 milligrams) of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraphs (c) and (d) of this section.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of sample
Amoxicillin trihydrate	None	Distilled water	1.0 mg.
Ampicillin	do	do	1.25 mg.
Ampicillin sodium	do	1	1.25 mg.
Ampicillin trihydrate	do	Distilled water	125 mg.

Antibiotic	Initial solvent	Diluent (solution number as listed in § 436.101(a))	Final concentration in units or milligrams of activity per milliliter of sample
Bacampicillin hydrochloride	None	.....do	1.25 mg.[2]
Cephalexin	.....do	.....do	2 mg.
Cephaloridine	.....do	.....do	2 mg.
Cephalothin sodium	.....do	.....do	2 mg.
Cephapirin sodium	.....do	.....do	2 mg.
Cloxacillin sodium monohydrate	.....do	.....do	1.25 mg.
Cloxacillin sodium monohydrate	.....do	.....do	1.25 mg.
Dicloxacillin sodium monohydrate	.....do	1	1.25 mg.
Cyclacillin	.....do	Distilled water	1.0 mg.
Methicillin sodium monohydrate	.....do	1	1.25 mg.
Mezlocillin	.....do	Distilled water	2.0 mg.
Nafcillin sodium monohydrate	.....do	1	1.25 mg.
Oxacillin sodium monohydrate	.....do	1	1.25 mg.
Penicillin G benzathine blank solution	.....do	Distilled water	2,000 units.
Penicillin G benzathine inactivated solution	.....do	1*N* NaOH	2,000 units.[1]
Penicillin G potassium	.....do	1	2,000 units.
Penicillin G procaine	2 ml methyl alcohol	1	2,000 units.
Penicillin G sodium	None	1	2,000 units.
Penicillin V	2 ml methyl alcohol	1	2,000 units.
Penicillin V potassium	.....do	1	2,000 units.

[1] Allow to stand in 1*N* NaOH for 15 minutes before assaying.
[2] The final concentration of bacampicillin hydrochloride is calculated in milligrams of ampicillin activity per milliliter of sample. The ampicillin working standard is used for the assay of bacampicillin hydrochloride.

(3) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested by diluting to the concentration prescribed in the table in paragraph (b)(2) of this section and proceed as described in paragraphs (c) and (d) of this section.

(c) *Inactivated sample and standard solutions.* (1) Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks.

(2) Add 2.0 milliliters of 1*N* sodium hydroxide, except if the sample has been diluted in 1*N* sodium hydroxide, and allow to stand at room temperature for 15 minutes.

(3) Add 2.0 milliliters of 1.2*N* hydrochloric acid.

(4) Add 10.0 milliliters of 0.01*N* iodine solution, stopper, allow to stand at room temperature for 15 minutes, and proceed as directed in paragraph (e) of this section.

(d) *Blank determination.* Transfer 2.0 milliliters each of the sample and the appropriate working standard solutions to glass-stoppered Erlenmeyer flasks. Add 10.0 milliliters of 0.01*N* iodine solution and immediately proceed as directed in paragraph (e) of this section.

(e) *Titration procedure.* Titrate the excess iodine using 0.01*N* sodium thiosulfate. Toward the end of the titration, add 1 drop of the starch iodide paste. Continue the titration by the addition of 0.01- to 0.02-milliliter portions of 0.01*N* sodium thiosulfate, shaking vigorously after each addition. The endpoint is reached when the blue color of the starch-iodine complex is discharged. Calculate the antibiotic content as described in paragraph (f) of this section.

(f) *Calculations*—(1) *F factor determination.* Using the appropriate working standard for the particular antibiotic to be tested, assay the standard as directed in this section. Calculate the F factor (the units of micrograms of activity equivalent of each milliliter of 0.01*N* sodium thiosulfate consumed) by means of the following formula:

$$F = \frac{W_s \times P}{V_s}$$

where:

W_s=Actual weight in milligrams of standard in the 2.0 milliliters titrated;

P=Potency of the working standard in units or micrograms per milligram;

V_s=Milliliters of sodium thiosulfate used in the working standard blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated working standard solution (the difference is the equivalent of the number of milliliters of 0.01*N* iodine absorbed by the inactivated standard).

(2) *Bulk antibiotic.* Calculate the potency of the sample in units or micrograms per milligram by means of the following formula:

$$\frac{V_u \times F}{W_u}$$

where:

V_u=Milliliters of sodium thiosulfate used in the sample blank determination minus the milliliters of sodium thiosulfate used in the titration of the inactivated sample solution (the difference is the equivalent of the number of milliliters of 0.01*N iodine absorbed by the inactivated sample);*

W_u=Actual weight in miligrams of sample in the 2.0 milliliters titrated.

(3) *Finished products.* Calculate the potency of the sample in units or milligrams by means of the appropriate one of the following formulas:

$$\text{Units of antibiotic per dose or item} = \frac{V_u \times F \times d}{2n}$$

$$\text{Milligrams of antibiotic per dose or item} = \frac{V_u \times F \times d}{n \times 2{,}000}$$

where:

d=Dilution factor for the sample;

n=Number of doses or items in the sample assayed.

[39 FR 18944, May 30, 1974, as amended at 39 FR 34032, Sept. 23, 1974; 42 FR 59856, Nov. 22, 1977; 44 FR 10378, Feb. 20, 1979; 46 FR 2980, Jan. 13, 1981; 46 FR 25602, May 8, 1981; 46 FR 46312, Sept. 18, 1981; 46 FR 58298, Dec. 1, 1981; 46 FR 61072, Dec. 15, 1981; 49 FR 6091, Feb. 17, 1984]

§ 436.205 Hydroxylamine colorimetric assay.

(a) *Reagents*—(1) *Hydroxylamine hydrochloride solution.* Dissolve 350 grams of hydroxylamine hydrochloride in sufficient distilled water to make 1 liter.

(2) *Buffer.* Dissolve 173 grams of sodium hydroxide and 20.6 grams of sodium acetate in sufficient distilled water to make 1 liter.

(3) *Neutral hydroxylamine.* Mix 1 volume each of hydroxylamine hydrochloride solution described in paragraph (a)(1) of this section and the buffer described in paragraph (a)(2) of this section. Check the pH and if necessary adjust to pH 7.0±0.1 by adding an additional amount of one of the components. To 1 volume of this neutralized solution add 8 volumes of distilled water and 2 volumes of 95 percent ethanol. This solution should be used for 1 day only.

(4) *Ferric ammonium sulfate.* Dissolve 272 grams of ferric ammonium sulfate in a mixture of 26 milliliters of concentrated sulfuric acid and sufficient distilled water to make 1 liter. This reagent may be used for 1 week when stored in a brown bottle at room temperature.

(b) *Preparation of working standard solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic working standard. Dissolve and dilute an accurately weighed portion to the specified final concentration and proceed as directed in paragraph (d) of this section.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of standard solution
Amoxicillin	Distilled water	1.0
Ampicillin	.....do	1.25
Cefazolin [1]		1.0
Cephaloridine	Distilled water	1.0
Cephalothin	.....do	2.0
Cephapirin	.....do	1.0
Cloxacillin	1	1.25
Cyclacillin	Distilled water	1.25
Dicloxacillin	1	1.25
Methicillin	1	1.25
Nafcillin	1	1.25
Oxacillin	1	1.25
Penicillin G	1	1.25
Penicillin G procaine	17	2.0
Penicillin V Potassium	1	1.25

[1] To prepare the working standard solution, proceed as directed in the individual section of the antibiotic drug regulation in this chapter for the antibiotic to be tested.

(c) *Preparation of sample solutions.* From the following table, select the diluent and final concentration as listed for each antibiotic. Dissolve an accurately weighed portion of the sample, dilute to the appropriate final concentration, and proceed as directed in paragraph (d) of this section; if the product is packaged for dispensing, dilute an aliquot of the stock solution (prepared as described in the individual monograph) to the appropriate concentration and then proceed as directed in paragraph (d) of this section.

Antibiotic	Diluent (solution number as listed in § 436.101(a))	Final concentration in milligrams per milliliter of sample
Amoxicillin trihydrate	Distilled water	1.0
Ampicillin	do	1.25
Ampicillin sodium	1	1.25
Ampicillin trihydrate	Distilled water	1.25
Bacampicillin hydrochloride	do	[1] 1.2
Cefazolin sodium	1	1.0
Cephaloridine	Distilled water	1.0
Cephalothin sodium	do	2.0
Cephapirin sodium	do	1.0
Cloxacillin sodium monohydrate.	1	1.25
Cyclacillin	Distilled water	1.25
Dicloxacillin sodium monohydrate.	do	1.25
Methicillin sodium monohydrate.	1	1.25
Nafcillin sodium monohydrate	1	1.25
Oxacillin sodium monohydrate	1	1.25
Penicillin G potassium	1	1.25
Penicillin G procaine	17	2.0
Penicillin G sodium	1	1.25
Penicillin V	17	1.25
Penicillin V potassium	1	1.25

[1] The final concentration of bacampicillin hydrochloride is calculated in milligrams of ampicillin per milliliter of sample. The ampicillin working standard is used for the assay of bacampicillin hydrochloride.

(d) *Procedure.* Using a volume of from 1 to 2 milliliters of standard or sample solution, add an equal volume of water and mix. Add the following reagents in the specified volumetric proportions with respect to the sample or standard solutions: Add 1.25 volumes of neutral hydroxylamine reagent and allow to react for 5 minutes. Add 1.25 volumes of ferric ammonium sulfate reagent, mix, and after 3 minutes determine the absorbance of the resulting solution at the wavelength of 480 millimicrons, using a suitable spectrophotometer and a reagent blank prepared by treating a volume of water in the same manner as the standard or sample solution. The time elapsed after the addition of the ferric ammonium sulfate reagent and the reading of the absorbance must be precisely the same (within 10 seconds) for each solution. Calculate the potency of the sample in units or micrograms per milligram as follows:

$$\text{Units or micrograms per milligram of sample} = \frac{A_1 \times \text{Potency (in units or micrograms per milliliter of standard solution)}}{A_2 \times \text{Milligrams of sample per milliliter of sample solution}}$$

A_1=Absorbance of sample solution.
A_2=Absorbance of standard solution.

[39 FR 18944, May 30, 1974, as amended at 39 FR 34032, Sept. 23, 1974; 39 FR 44012, Dec. 20, 1974; 42 FR 59856, Nov. 22, 1977; 44 FR 10378, Feb. 20, 1979; 45 FR 16474, Mar. 14, 1980; 46 FR 2981, Jan. 13, 1981; 46 FR 25602, May 8, 1981; 46 FR 61072, Dec. 15, 1981; 49 FR 34350, Aug. 30, 1984]

§ 436.206 Test for metal particles in ophthalmic ointments.

(a) *Procedure.* Extrude the contents of each of 10 tubes as completely as practicable into separate, clear, glass Petri dishes (60 millimeters in diameter), cover the dishes, and heat to 80° C. to 85° C. for at least 2 hours or until the ointment has melted completely and evenly in the dishes. A higher temperature of 100° C.±2° C. may be used if necessary to allow adequate settling of metal particles. Allow the ointment to cool to room temperature without agitation. Invert each Petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eye-piece micrometer disc which has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45° angle. Examine the entire bottom of the Petri dish for metal particles. By varying the intensity of the illuminator from above, such metal particles are recognized by their characteristic reflection of light. Count the total number of metal particles exceeding 50 microns in any single dimension.

(b) *Evaluation.* The batch is acceptable if (1) a total of not more than 50 such particles is found in 10 tubes; and (2) not more than one tube is found to contain more than eight such particles. If the batch fails the above test, repeat

the test on 20 additional tubes of ointment. The total number of metal particles exceeding 50 microns in any single dimension from the 30 tubes tested shall not exceed 150, with not more than three tubes containing more than eight such particles.

[39 FR 18944, May 30, 1974; 40 FR 11869, Mar. 14, 1975]

§ 436.207 Residue on ignition.

Use the method specified in the individual section for each antibiotic.

(a) *Method 1.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add 2 milliliters of nitric acid and 5 drops of sulfuric acid to the contents of the crucible and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the sulfated ash content.

(b) *Method 2.* Proceed as directed in paragraph (a) of this section, except use 2 milliliters of sulfuric acid and do not use the nitric acid.

§ 436.208 Heavy metals determination.

(a) *Reagents*—(1) *Ammonia solution.* Prepare an aqueous solution containing not less than 9 grams and not more than 10 grams of ammonia (NH_3) per 100 milliliters.

(2) *6 percent acetic acid.* Dilute 60 milliliters of glacial acetic acid with sufficient water to give a solution of 1,000 milliliters.

(3) *Hydrogen sulfide solution.* Prepare a saturated solution of hydrogen sulfide by passing hydrogen sulfide into cold water for a sufficient time. It is suitable if it produces an immediate copious precipitate when added to an equal volume of 1*N* ferric chloride. Prepare a fresh hydrogen sulfide solution each time a heavy metals test is to be performed.

(4) *Lead nitrate stock solution.* Dissolve 159.8 milligrams of lead nitrate with 100 milliliters of 0.15*N* nitric acid, and dilute with water to a volume of 1,000 milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(5) *Standard lead solution.* Dilute a 10-milliliter aliquot of the lead nitrate stock solution to 100 milliliters with water. This solution must be freshly prepared each time a heavy metals test is performed. One milliliter of this standard lead solution represents a lead level of 10 parts per million in a 1.0-gram sample or 20 parts per million in a 0.5-gram sample.

(b) *Preparation of the sample.* Use the sulfated ash obtained as described in § 436.207(a). If the heavy metal limit is greater than 30 parts per million, the sulfated ash may be obtained from a 0.5-gram sample. Add 2 milliliters of hydrochloric acid to the sulfated ash and slowly evaporate to dryness on a steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 milliliters of hot water, and digest by heating on the steam bath for 2 minutes. After cooling to room temperature, add ammonia solution dropwise until a pH of 7.2 is reached, then add 2 milliliters of 6 percent acetic acid. Filter the solution, if necessary, and wash the crucible and the filter with about 10 milliliters of water. Combine the washings with the filtrate and dilute to exactly 25 milliliters with water.

(c) *Procedure.* Prepare a series of five standard lead solutions, in increments of 10 parts per million, in which the solution of lowest concentration contains 20 parts of lead per million less than the maximum limit of heavy metals permitted for the sample. Transfer the necessary quantities of standard lead solution described in paragraph (a)(5) of this section directly into metal-free 50-milliliter Nessler tubes of uniform diameter, add 2 milliliters of 6 percent acetic acid to each, and adjust each to a final volume of 25 milliliters with water. Transfer the 25-milliliter solution of the sample described in paragraph (b) of this section to another Nessler tube. Add 10 milliliters of hydrogen sulfide solution to each standard and sample solution, mix well, and allow to stand for 10 minutes. View downward over a white surface; the color of the solution of the sample should be no darker than the standard

that contains the lead equivalent of the heavy metals limit of the test.

§ 436.209 Melting range or temperature.

(a) *Apparatus.* Melting range apparatus consists of a glass container for a bath of colorless fluid, a suitable stirring device, an accurate thermometer, and a controlled source of heat. Any apparatus or method of equal accuracy may be used. The accuracy should be checked periodically by use of melting point standards, preferably those that melt near the expected melting range of the product to be tested. The bath fluid is selected with a view to the temperature required, but light paraffin is used generally and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still 2 centimeters above the bottom of the bath.

(b) *Sample preparation.* If necessary, reduce the sample to a fine powder and store it in a desiccator over sulfuric acid for 24 hours. If a method for loss on drying is included in the section for the antibiotic to be tested, a sample dried by that method may be used.

(c) *Test procedure.* Use a capillary glass tube about 10 centimeters long and 0.8 to 1.2 millimeters internal diameter with the wall 0.2 to 0.3 millimeter in thickness. Charge the tube with a sufficient amount of the dry power to form a column 2.5 to 3.5 millimeters high from the sealed end when packed down as closely as possible by moderate tapping on a solid surface. Heat the bath until a temperature 10°±1° C. below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3°±0.5° C. per minute until melting is completed. The temperature at which the column of the sample is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of melting or the melting point.

[39 FR 18944, May 30, 1974, as amended at 41 FR 24883, June 21, 1976]

§ 436.210 Specific rotation.

(a) *Test procedure.* The appropriate solvent, test concentration, and polarimeter tube length are specified in the section for each antibiotic to be tested. Accurately weigh the sample to be tested in a glass-stoppered volumetric flask, dissolve in the appropriate solvent, and dilute to the specified test concentration at 25° C. Maintain the solution at 25° C. and transfer to the appropriate polarimeter tube. Determine the angular rotation of both solvent and sample solution in a suitable polarimeter, using a sodium light source or a white light source with a 589.3-millimicron filter. The zero correction is the average of the blank readings and is subtracted from the average observed rotation of the sample solution if the two figures are of the same sign, or is added if they are opposite in sign, to give the corrected angular rotation of the sample solution. The determination must be completed within one-half hour from the time the solution is prepared.

(b) *Calculations.* Determine the specific rotation. [α], by the following formula:

$$[a]t_x = \frac{100a}{lc}$$

where:

a=The corrected angular rotation of the sample solution in degrees at temperature *t* using a light source of a wavelength of *x* millimicrons;

l=The length of the polarimeter tube in decimeters;

c=The concentration of the solution expressed as number of grams of substance in 100 milliliters of solution.

§ 436.211 Identity test by infrared spectrophotometry.

(a) *Apparatus*—(1) *Spectrophotometer.* A suitable spectrophotometer capable of recording the infrared absorption spectrum in the 2 to 15 micron range.

(2) *Hydraulic press.* A 30-ton hydraulic press with 12-inch square platens.

(b) *Sample preparation methods.* Use the sample preparation method specified in the individual section for each antibiotic.

(1) *Potassium bromide discs.* Quantities of materials specified are for a 13-millimeter die. Appropriate adjustments

should be made in the quantities of materials when dies of other sizes are used. To prepare a 1.0 percent mixture weigh approximately 2 milligrams of the sample and mix thoroughly with 200 milligrams of dried potassium bromide (infrared spectrophotometric quality). For a 0.5 percent potassium bromide mixture, use 1 milligram of sample. For a 0.25 percent potassium bromide mixture, use 0.5 milligram of sample. A mortar and pestle, a ball mill, or other suitable mixing device may be used. Transfer the uniformly milled mixture to the die, evacuate gradually while raising the pressure to 3,000 pounds per square inch until evacuation is complete, then raise the pressure to 16,000 pounds per square inch, and hold that pressure for 2 to 3 minutes. Release the pressure, dismantle the die, and recover the potassium bromide disc. Mount the disc in a suitable holder and proceed as directed in paragraph (c) of this section.

(2) *Mineral oil mull.* Weigh approximately 20 milligrams of the sample into an agate mortar and add 2 drops of mineral oil. Triturate thoroughly with a pestle until a uniform consistency is obtained. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles. Proceed as directed in paragraph (c) of this section.

(3) *1 percent solution.* Prepare a 1 percent solution of the sample in chloroform and use 1.0 millimeter matched absorption cells. Proceed as directed in paragraph (c) of this section.

(c) *Procedure.* Place the sample, prepared as directed in paragraph (b) of this section, in the spectrophotometer. Determine the infrared absorbance spectrum between the wavelengths of 2 to 15 microns. To be suitable the spectrum should have a transmittance of between 20 and 70 percent at most of the wavelengths showing significant absorption. Compare the spectrum to that of an authentic sample of the same antibiotic prepared in an identical manner. To pass the infrared identity test, the absorption spectrum of the sample should compare qualitatively with that of the authentic sample.

§ 436.212 Disintegration test.

(a) *Apparatus*—(1) *Basket-rack assembly.* The basket-rack assembly consists of 6 open-ended glass tubes, each 7.75±0.25 centimeters long and having an inside diameter of approximately 21.5 millimeters and a wall approximately 2 millimeters thick; the tubes are held in a vertical position by two plastic plates, each about 9 centimeters in diameter and 6 millimeters in thickness, with six holes, each about 24 millimeters in diameter, equidistant from the center of the plate and equally spaced from one another. Attached by screws to the undersurface of the lower plate is 10-mesh No. 23 (0.025 inch) W. and M. gauge woven stainless steel wire cloth. The glass tubes and the upper plastic plate are secured in position at the top by means of a stainless steel plate, about 9 centimeters in diameter and 1 millimeter in thickness, having six perforations each about 20 millimeters in diameter, which coincide with those of the upper plastic plate and the upper open ends of the glass tubes. A central shaft about 8 centimeters in length, the upper end of which terminates in an eye through which a string or wire may be inserted, is attached to the stainless steel plate. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plastic plates and the steel plate. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained.

(2) *Disks.* Each tube is provided with a slotted and perforated cylindrical disk 9.5±0.15 millimeters thick and 20.7±0.15 millimeters in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five 2-millimeter holes extend between the ends of the cylinder, one of the holes being through the cylinder axis and the others parallel with it and equally spaced on a 6-millimeter radius from it.

Equally spaced on the sides of the cylinder are four notches that form V-shaped planes perpendicular to the ends of the cylinder. The dimensions of each notch are such that the openings on the bottom of the cylinder are 1.60 millimeters square and those on the top are 9.5 millimeters wide and 2.55 millimeters deep. All surfaces of the disk are smooth.

(3) *Raising and lowering device.* Use a device for raising and lowering the basket in the immersion fluid at a constant rate between 28 and 32 cycles per minute through a distance of not less than 5 centimeters and not more than 6 centimeters.

(b) *Immersion fluids.* During the performance of the tests all immersion fluids are maintained at a temperature of 37°±2° C. by using a thermostatically controlled water bath.

(1) Distilled water.

(2) Simulated gastric fluid: Dissolve 2.0 grams of sodium chloride and 7.0 milliliters of hydrochloric acid in about 500 milliliters of water. Dissolve 3.2 grams of pepsin in this solution and add sufficient water to make 1,000 milliliters. This solution has a pH of about 1.2.

(3) Simulated intestinal fluid: Dissolve 6.8 grams of monobasic potassium phosphate in 250 milliliters of water, mix and add 190 milliliters of 0.2*N* sodium hydroxide and 400 milliliters of water. Add 10.0 grams of pancreatin, mix, and adjust the resulting solution with 0.2*N* sodium hydroxide to a pH of 7.5±0.1. Dilute to 1,000 milliliters.

(c) *Immersion vessel.* Use a suitable vessel, such as a 1-liter beaker.

(d) *Operation.* Add enough immersion fluid to the immersion vessel so that when the basket-rack assembly is placed on the raising and lowering device at the highest point of the upward stroke, the wire mesh remains at least 2.5 centimeters below the surface of the fluid and descends to not less than 2.5 centimeters from the bottom of the immersion vessel.

(e) *Procedure*—(1) *Uncoated or filmcoated tablets.* Place one tablet into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluid as the immersion fluid. At the end of the time limit specified in the individual section for the particular antibiotic tablet being tested, lift the basket from the fluid and observe the tablets.

(2) *Plain-coated tablets.* Place one tablet in each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using simulated gastric fluids as the immersion fluid. After 30 minutes, lift the basket from the fluid and observe the tablets. If the tablets have not disintegrated completely, substitute simulated intestinal fluid as the immersion fluid and continue the test for a total period of time (including previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(3) *Enteric-coated tablets.* Place one tablet in each of the six tubes of the basket and operate the apparatus, using simulated gastric fluid as the immersion fluid. One hour later, lift the basket from the fluid and observe the tablets. If the tablets show no distinct evidence of dissolution or disintegration, add a disk to each tube and operate the apparatus, using simulated intestinal fluid as the immersion fluid, for a total period of time (including the previous immersion in simulated gastric fluid) equal to the time limit specified in the individual section for the particular antibiotic tablet being tested. Lift the basket and observe the tablets.

(4) *Pastilles.* Place one pastille into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using distilled water as the immersion fluid. At the end of the time limit specified in the individual section for the particular antibiotic pastille being tested, lift the basket from the fluid and observe the pastilles.

(5) *Capsules.* Place one capsule into each of the six tubes of the basket, add a disk to each tube, and operate the apparatus, using distilled water as the immersion fluid. At the end of the time limit specified in the individual section for the capsules being tested, lift the basket from the fluid and observe the capsules.

(f) *Evaluation.* Complete disintegration is defined as the state in which any residue of the tablet, pastille, or

capsule (except fragments of the insoluble coating) remaining on the screen is a soft mass having no palpably firm core. The tablets, pastilles, or capsules pass the disintegration test if all of the units tested disintegrate completely under the conditions and time specified in the individual section for the antibiotic tablet, pastille, or capsule being tested. If one or two tablets, pastilles, or capsules fail to disintegrate completely, repeat the test on 12 additional tablets, pastilles, or capsules. The tablets, pastilles, or capsules pass the disintegration test if not less than 16 of the total 18 tested disintegrate completely. Enteric coated tablets fail the disintegration test if they show any distinct evidence of dissolution or disintegration after 1 hour immersion in simulated gastric fluid.

[39 FR 18944, May 30, 1974, as amended at 52 FR 4617, Feb. 13, 1987; 55 FR 19873, May 14, 1990]

§ 436.213 Nonaqueous titrations.

(a) *Equipment*—(1) *Apparatus.* Use a closed system consisting of a suitable titrimeter equipped with a potentiometer, an automatic burette, a chart recorder, and a glass calomel combinatin electrode (with saturated methanolic potassium chloride as the electrolyte).

(2) *Titration vessel.* Use a 100-milliliter tall form beaker without a spout.

(b) *Reagents*—(1) Methyl alcohol, reagent grade, anhydrous.

(2) Dimethylsulfoxide, A.C.S., reagent grade.

(3) Glacial acetic acid, A.C.S., reagent grade.

(4) Lithium methoxide reagent: 0.02*N* lithium methoxide in methyl alcohol, standardized against primary grade benzoic acid.

(5) Perchloric acid reagent: 0.02*N* perchloric acid in glacial acetic acid, standardized against primary grade potassium acid phthalate.

(c) *Preparation of sample solutions.* Select the weight of the sample and the solvent listed for each antibiotic. Transfer the accurately weighed sample to a titration vessel. Add the appropriate solvent, cover, and stir magnetically until the sample is dissolved. Proceed as directed in paragraph (e) of this section, using the procedure or procedures specified in the individual section for each antibiotic.

Antibiotic	Weight in milligrams of sample	Solvent
Amoxicillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Amoxicillin-base titration.	100	50 milliliters glacial acetic acid.
Ampicillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Ampicillin-base titration	100	50 milliliters glacial acetic acid.
Ampicillin sodium-base titration.	50	Do.
Cephaloglycin-base titration.	50	Do.
Cephapirin sodium-base titration.	50	50 milliliters glacial acetic acid.
Cyclacillin-acid titration	100	20 milliliters dimethylsulfoxide and 30 milliliters methyl alcohol.*
Cyclacillin-base titration	100	50 milliliters glacial acetic acid.
Tobramycin-base titration.	30	50 ml glacial acetic acid.

*The methyl alcohol is added after the sample has dissolved in dimethylsulfoxide.

(d) *Blank determination.* Place the same volume of solvent used to prepare the sample solution into a titration vessel and proceed as directed in paragraph (e) of this section, using the procedure or procedures specified in the individual section for each antibiotic.

(e) *Titration procedures*—(1) *Acid titration.* Equilibrate the electrode by soaking it overnight in the solvent used for preparing the sample solution. Start the magnetic stirrer and titrate the sample solution with the lithium methoxide reagent. Record the change in potential of the solution with the addition of the titrant. Determine the number of milliliters of reagent consumed at neutralization (the inflection point of the titration curve). Calculate the antibiotic content as directed in the individual section.

(2) *Base titration.* Proceed as directed in paragraph (e)(1) of this section, except use the perchloric acid reagent as the titrant and calculate the antibiotic

content as directed in the individual section.

[39 FR 18944, May 30, 1974, as amended at 40 FR 22251, Apr. 22, 1975; 40 FR 23725, June 2, 1975; 40 FR 57797, Dec. 12, 1975; 46 FR 2981, Jan. 13, 1981]

§ 436.214 Heat stability.

Store an accurately weighed portion of the sample of approximately 30 milligrams in an unstoppered 50-milliliter Erlenmeyer flask for 4 days in an electric oven at 100° C±1° C. At the end of this period, remove the flask from the oven and allow to cool in a desiccator. Accurately weigh an unheated portion of the original sample of approximately 30 milligrams. Assay both the heated and unheated samples for potency as directed in § 436.204 or § 436.205 of this chapter. Determine the percent loss from the difference in potency between the unheated original sample and the heat-treated sample.

§ 436.215 Dissolution test.

(a) *Equipment.* Use either Apparatus 1 or 2 as described in the United States Pharmacopeia XXI dissolution test.

(b) *Procedure.* For each dosage form listed in the table in this paragraph select the appropriate dissolution medium, rotation rate, sampling time, and apparatus, and proceed as set forth in either Apparatus 1 or 2 methodology of the United States Pharmacopeia XXI dissolution test. Determine the amount of drug substance dissolved by performing the assay described in paragraph (c) of this section. The amount of dissolution medium removed for sampling purposes may be disregarded if the amount removed is not more than 15 milliliters. If more than 15 milliliters is removed, then correct for the volume removed.

[42 FR 59856, Nov. 22, 1977]

Dosage form	Dissolution medium	Rotation rate [1]	Sampling time(s)	Apparatus
Amoxicillin trihydrate and clavulanate potassium chewable tablets..	900 mL distilled water	75	30 min	2
Amoxicillin trihydrate and clavulanate potassium tablets.	.....do	75	.....do	2
Azithromycin capsules.	900 mL 0.10 *M* sodium phosphate buffer, pH 6.0, 0.1 mg/mL trypsin.	100	45 min	2
Bacampicillin hydrochloride tablets.	.....do	75	.....do	2
Cefadroxil hemihydrate capsules.	900 mL distilled water	100	45 min	1
Cefadroxil hemihydrate tablets.	900 mL distilled water	50	30 min	2
Cefixime tablets	900 mL 0.05 *M* potassium phosphate buffer, pH 7.2.	100	45 min	1
Cefpodoxime proxetil tablets	900 mL pH 3.0 glycine buffer	75	30 min	2
Cefprozil tablets.	900 mL purified water	100	45 min	1
Cefuroxime axetil for oral suspension	900 mL Sorenson's Modified Phosphate Buffer, pH 7.0.	50	30 min	2
Cefuroxime axetil tablets.	900 mL 0.07*N* hydrochloric acid	55	15 min. and 45 min	2
Cephalexin hydrochloride monohydrate tablets..	900 mL distilled water	150	45 min	1
Cephradine dihydrate capsules.	900 mL 0.12*N* hydrochloric acid	75	60 min.	2
Clarithromycin tablets.	900 mL 0.10 *M* sodium acetate buffer, pH 5.0.	50	30 min.	2
Doxycycline hyclate tablets.	900 mL distilled water	75	60 min and 90 min	2
Doxycycline monohydrate hydrochloric acid capsules..	900 mL 0.1*N* hydrochloric acid.	75	60 min	2
Erythromycin particles in tablets	900 mL 0.05*M* potassium phosphate buffer, pH 6.8.	75	45 min.	2
Loracarbef capsules.	900 mL distilled water.	50	30 min	2
Oxytetracycline hydrochloride capsules..	900 mL distilled water	75	30 min and 60 min	2
Rifabutin capsules	900 mL 0.01 *N* hydrochloric acid	100	45 min	1
Tetracycline hydrochloride capsules (except 500-mg)..	.....do	75	.....do	2
Tetracycline hydrochloride capsules (500-mg)..	.....do	75	30 min, 60 min, and 90 min	2
Tetracycline hydrochloride tablets.	.....do	75	30 min and 60 min	2
Vancomycin hydrochloride capsules.	900 mL distilled water.	100	45 min	1

[1] Rotation rate of basket or paddle stirring element (revolutions per minute).

(c) *Antibiotic drug content*—(1) *Tetracycline hydrochloride*—(i) *Preparation of working standard solution.* Accurately weigh 20 to 30 milligrams of tetracycline hydrochloride working standard into a suitable-sized volumetric flask. Dissolve and dilute to volume with water. Further dilute an accurately measured portion with distilled water to obtain a known concentration of 0.01 to 0.02 milligram of tetracycline hydrochloride per milliliter.

(ii) *Preparation of sample solutions.* Dilute an accurately measured portion of the sample with sufficient distilled water to obtain a concentration of 0.01 to 0.02 milligram of tetracycline hydrochloride per milliliter (estimated).

(iii) *Procedure.* Using a suitable spectrophotometer and water as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 276 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(iv) *Calculation.* Determine the total amount of tetracycline hydrochloride dissolved as follows:

$$T = \frac{A_u \times c \times d \times 900^*}{A_s}$$

where:

T=Total milligrams of drug dissolved;
A_u=Absorbance of sample;
c=Concentration of standard in milligrams;
d=Dilution factor of sample filtrate;
A_s=Absorbance of standard.

*If more than 15 mL of dissolution medium is removed, correct for the volume removed.

(2) *Oxytetracycline hydrochloride; preparation of working standard-solution.* (i) Accurately weigh 30 milligrams of oxytetracycline-base working standard into a suitable-sized volumetric flask. Add 5 milliliters of 0.1*N* hydrochloric acid and swirl the flask to dissolve oxytetracycline base. Dilute an accurately measured portion with distilled water to obtain a known concentration of 0.01 to 0.02 milligram of oxytetracycline per milliliter.

(ii) Proceed as directed in paragraphs (c)(1) (ii), (iii), and (iv) of this section except measure the absorbance at the absorption peak at approximately 273 nanometers.

(3) *Doxycycline hyclate.* Proceed as directed in paragraph (c)(1) of this section, except use the doxycycline working standard.

(4) *Bacampicillin hydrochloride.* Use the ampicillin working standard as the standard of comparison and assay for ampicillin content by either of the following methods.

(i) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except dilute the working standard to a final concentration of 0.3 milligram of ampicillin per milliliter and use the sample solution as it is removed from the dissolution vessel without further dilution.

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except:

(*a*) *Buffer.* In lieu of the buffer described in § 442.40(b)(1)(ii)(*b*)(*2*) of this chapter, use the buffer prepared as follows: Dissolve 200 grams of primary standard tris (hydroxymethyl) aminomethane in sufficient distilled water to make 1 liter. Filter before use.

(*b*) *Preparation of the working standard solution.* Dissolve and dilute an accurately weighed portion of the ampicillin working standard with sufficient distilled water to obtain a final concentration of 0.3 milligram of ampicillin per milliliter;

(*c*) *Sample solution.* Use the sample solution as it is removed from the dissolution vessel without further dilution; and

(*d*) *Calculations.* Determine the total amount of ampicillin dissolved as follows:

$$T = \frac{(A_u)(c)(d)(900^*)}{A_s}$$

where:

T=Total milligrams of ampicillin equivalent dissolved;
A_u=Absorbance of sample;
c=Concentration of working standard solution in milligrams per milliliter;
d=Dilution factor of sample filtrate;
A_s=Absorbance of standard.

*If more than 15 mL of dissolution medium is removed, correct for the volume removed.

(5) *Cephradine dihydrate*—(i) *Preparation of working standard solution.* Accurately weigh approximately 40 milligrams of cephradine working standard

into a suitable-sized volumetric flask. Dissolve and dilute to volume with 0.12*N* hydrochloric acid. Further dilute with a buffer solution (prepared by dissolving 27.2 grams of sodium acetate trihydrate in a mixture of 12 milliliters of glacial acetic acid and sufficient distilled water to make 2 liters) to obtain a known concentration of 0.01 to 0.03 milligram of cephradine per milliliter.

(ii) *Preparation of sample solution.* Filter the sample and dilute an accurately measured portion of the filtrate with sufficient buffer solution, described in paragraph (c)(5)(i) of this section, to obtain a concentration of 0.01 to 0.03 milligram of cephradine per milliliter (estimated).

(iii) Proceed as directed in paragraphs (c)(1) (iii) and (iv) of this section, except measure the absorbance at the absorption peak at approximately 262 nanometers.

(6) *Amoxicillin trihydrate.* Assay for the amoxicillin content as described in § 440.103d of this chapter, except use the sample as it is removed from the dissolution vessel.

(7) *Vancomycin hydrochloride.* Assay for the vancomycin content as described in § 436.105 of this chapter, except use the sample as it is removed from the dissolution test.

(8) *Erythromycin*—(i) *Preparation of working standard solution.* Accurately weigh approximately 140 milligrams of erythromycin working standard into a 250-milliliter volumetric flask and dissolve in 10 milliliters of methyl alcohol. Add water nearly to volume, mix, and allow the solution to cool. Dilute to volume with water and mix. On the day of use, dilute an accurately measured aliquot with water to obtain a known concentration of 0.28 milligram of erythromycin per milliliter (before adjusting for standard potency).

(ii) *Preparation of sample solution.* Dilute an accurately measured portion of the filtered sample with sufficient 0.05*M* potassium phosphate buffer, pH 6.8, to obtain a concentration of about 0.28 milligram of erythromycin per milliliter (estimated).

(iii) *Procedure.* Transfer 5.0-milliliter aliquots of the working standard solution and sample solution to 25-milliliter volumetric flasks and treat as follows: Add 2.0 milliliters of water, allow to stand for 5 minutes with intermittent swirling. Add 15.0 milliliters of 0.25*N* sodium hydroxide, dilute to volume with sufficient 0.05*M* potassium phosphate buffer, pH 6.8, and mix. Heat to 60 °C for 5 minutes and allow to cool. Using a suitable spectrophotometer and a blank (prepared as per the procedure above except that 2.0 milliliters of 0.5N sulfuric acid is substituted for the 2.0 milliliters of water) for each solution, determine the absorbance of each working standard and sample solution at the absorbance peak at approximately 236 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(iv) *Calculation.* Proceed as directed in paragraph (c)(1)(iv) of this section.

(9) *Cefuroxime axetil tablets and powder for oral suspension*—(i) *Preparation of working standard solution*—(*a*) *Cefuroxime axetil tablets.* Accurately weigh approximately 60 milligrams of cefuroxime axetil working standard into a suitable-sized volumetric flask. Dissolve in 5 milliliters of methanol and dilute to volume with 0.07*N* hydrochloric acid to obtain a known concentration equivalent to 0.01 to 0.02 milligram of cefuroxime activity per milliliter.

(*b*) *Cefuroxime axetil for oral suspension.* Accurately weigh approximately 15 milligrams of cefuroxime axetil working standard into a 100-milliliter volumetric flask. Dissolve in 5 milliliters of methanol and dilute to volume with Sorenson's Modified Phosphate Buffer, pH 7.0 (4.2 grams of sodium dihydrogen orthophosphate dihydrate and 14.3 grams of hydrogen disodium orthophosphate dodecahydrate per liter of water).

(ii) *Preparation of sample solution*—(*a*) *Cefuroxime axetil tablets.* Filter through a 0.45-micron filter and dilute an accurately measured portion of the filtrate with sufficient 0.07*N* hydrochloric acid to obtain a concentration equivalent to 0.01 to 0.02 milligram of cefuroxime activity per milliliter (estimated).

(*b*) *Cefuroxime axetil for oral suspension.* Filter the sample through an 8-micron filter. A coarse prefilter may be used to prevent clogging. Use the filtrate solution without further dilution.

(iii) *Procedure*—(*a*) *Cefuroxime axetil tablets*. Using a suitable spectrophotometer and 0.07*N* hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 280 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(*b*) *Cefuroxime axetil for oral suspension*. Using a suitable spectrophotometer and Sorenson's Modified Phosphate Buffer, pH 7.0 (4.2 grams of sodium dihydrogen orthophosphate dihydrate and 14.3 grams of hydrogen disodium orthophosphate dodecahydrate per liter of water) as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 280 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(iv) *Calculations*. Determine the total amount of cefuroxime activity dissolved as follows:

$$T = \frac{A_u \times c \times d \times 900}{A_s}$$

where:

T = Total milligrams of cefuroxime activity dissolved;

A_U = Absorbance of sample;

c = Cefuroxime activity of working standard solution in milligrams per milliliter;

d = Dilution factor of sample filtrate; and

A_s = Absorbance of standard.

(10) *Cefixime*—(i) *Preparation of working standard solution*. Accurately weigh approximately 25 milligrams of cefixime working standard into a 500-milliliter volumetic flask. Wet the powder with 0.5 milliliters of methanol, and dilute to volume with 0.05 *M* potassium phosphate buffer, pH 7.2 (prepared by dissolving 6.8 grams of monobasic potassium phosphate in distilled water to a volume of one liter. The pH is adjusted to 7.2 with 1.0*N* NaOH). Sonicate to assure dissolution and mix.

(ii) *Preparation of sample solution*. Forty-five minutes after the beginning of the rotation, withdraw and filter a portion of the solution. For the 400-milligram tablets, pipet 10.0 milliliters of the filtered sample solution into a 100-milliliter volumetric flask. For the 200-milligram tablets, pipet 10.0 milliliters of the filtered sample into a 50-milliliter volumetric flask. Dilute to volume with 0.05 *M* postassium phosphate buffer, pH 7.2.

(iii) *Procedure*. Proceed as directed in paragraphs (c)(1) (iii) and (iv) of this section, except measure the absorbance of the peak at approximately 320 nanometers using 0.05 *M* potassium phosphate buffer, pH 7.2 as the blank.

(11) *Cephalexin hydrochloride monohydrate*. Assay for cephalexin activity of the cephalexin hydrochloride monohydrate as directed in § 442.28 of this chapter, and use U.S.P. dissolution apparatus 1 (10 mesh basket). Use the sample as it is removed from the dissolution vessel.

(12) *Doxycycline monohydrate*. Proceed as directed in paragraph (c)(1) of this section, except use the doxycycline standard.

(13) *Clarithromycin*. Proceed as directed in § 452.50(b)(1) of this chapter except:

(i) *Dissolution medium*. Instead of the mobile phase described in § 452.50(b)(1)(i) of this chapter, use 0.10 *M* sodium acetate buffer prepared as follows: Weigh 13.6 grams of sodium acetate trihyrate into a container sufficient to hold 1 liter of solution. Dissolve the salt in 750 milliliters of distilled water. Adjust the pH of the solution to 5.0±0.05 with glacial acetic acid. Dilute to 1,000 milliliters with distilled water.

(ii) *Preparation of the standard and sample solutions*—(*a*) *Standard solution*. Dissolve (with shaking or sonication) an accurately weighed portion of the clarithromycin working standard, in sufficient methanol to obtain a solution having a known concentration of approximately 625 micrograms per milliliter of clarithromycin. Quantitatively transfer and dilute an aliquot of this solution with mobile phase (described in § 452.50(b)(1)(i) of this chapter) and mix to obtain a solution of known concentration of approximately 125 micrograms per milliliter of clarithromycin.

(*b*) *Sample solution*. Use the sample solution as it is removed from the dissolution vessel after diluting and mixing with mobile phase (described in § 452.50(b)(1)(i) of this chapter) 1:2 for

the 250-milligram tablet and 1:4 for the 500-milligram tablet.

(*c*) *Calculations.* Determine the total amount of clarithromycin activity dissolved as follows:

$$T = \frac{A_U \times c \times d \times 900}{A_s}$$

where:

T = Total milligrams of clarithromycin activity dissolved;

A_U = Area of the clarithromycin peak (at a retention time equal to that observed for the standard) in the chromatogram of the sample;

A_S = Area of the clarithromycin peak in the chromatogram of the clarithromycin standard;

c = Clarithromycin activity in the clarithromycin working standard solution in milligrams per milliliter; and

d = Dilution factor of sample filtrate.

(14) *Azithromycin.* Proceed as directed in § 452.60(b)(1) of this chapter, except:

(i) *Dissolution medium.* Dissolve 85.2 grams of sodium phosphate dibasic and dilute to volume with ultrapure deionized or high-performance liquid chromatographic-grade water in a stoppered 2-liter graduated cylinder. Dilute this entire solution in an appropriate, suitably sized container with 4 liters of ultrapure deionized or high-performance liquid chromatographic-grade water. Adjust the pH to 6.0±0.05 with concentrated hydrochloric acid (about 40.5 milliliters). Add 600 milligrams of trypsin and mix well.

(ii) *Preparation of the standard and sample solutions*—(*a*) *Standard solution.* Accurately weigh approximately 15 milligrams of the azithromycin working standard into a 50-milliliter volumetric flask. Add 25 milliliters of the dissolution medium and sonicate briefly. Dilute to volume with dissolution medium and mix well. Pipet 2.0 milliliters of this solution into a 25-milliliter volumetric flask and dilute to volume with the mobile phase described in § 452.60(b)(1)(i) of this chapter. Pipet 4.0 milliliters of this solution into a 25-milliliter volumetric flask and bring to volume with the mobile phase.

(*b*) *Sample solution.* Filter the sample solutions through a 0.45-micron filter before use. Pipet 2.0 milliliters of the filtered aliquot into a 25-milliliter volumetric flask and dilute to volume with the mobile phase described in § 452.60(b)(1)(i) of this chapter. Pipet 4.0 milliliters of this solution into another 25-milliliter volumetric flask and bring to volume with the mobile phase. The solution is stable at room temperature for 24 hours.

(*c*) *Calculations.* Determine the percent of azithromycin dissolved as follows:

$$\text{Percent azithromycin dissolved} = \frac{A_U \times P_s \times D_F \times 100}{A_s \times W_u}$$

where:

A_U =Area of the azithromycin peak (at a retention time equal to that observed for the standard) in the chromatogram of the sample;

A_S =Area of the azithromycin peak in the chromatogram of the azithromycin standard;

P_S =Azithromycin activity in the azithromycin working standard solution in micrograms per milliliter;

$$DF = \text{Dilution factor} = \frac{900^1 \times 25 \times 25}{2 \times 4}$$

[1] If more than 15 milliliters of dissolution medium are removed, correct for the volume removed; and

W_U =Theoretical azithromycin content (mg) of capsule.

(15) *Cefprozil.* Proceed as directed in § 442.80(b)(1) of this chapter except:

(i) *Sample solutions.* Filter the sample solutions through a 0.45-micron filter before use. Use the sample solution as it is removed from the dissolution vessel without further dilution for the 250-milligram tablet; prepare the sample solution for the 500-milligram tablet by diluting a 5-milliliter aliquot of the filtered solution to volume in a 10-milliliter volumetric flask with distilled water.

(ii) *Calculations.* Determine the total percent of cefprozil dissolved as follows:

$$\text{Total percentage dissolved} = \frac{(\text{mg cefprozil (Z) dissolved} + \text{mg cefprozil (E) dissolved})}{\text{label claim}}$$

$$\text{Milligrams of cefprozil (Z) or cefprozil (E) dissolved} = \frac{A_U \times c \times d \times 900}{A_s}$$

where:

A_U = Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the cefprozil (Z) or cefprozil (E) standard;

c = Concentration of the cefprozil (Z) or cefprozil (E) working standard solution in milligrams per milliliter; and

d = Dilution factor of the sample filtrate.

(16) *Loracarbef*—(i) *Preparation of the working standard solution.* Accurately weigh approximately 110 milligrams of the loracarbef working standard into a suitable-sized volumetric flask. Dissolve and dilute to volume with water. Further dilute an accurately measured portion with distilled water to obtain a known concentration of 0.02 milligram of loracarbef activity per milliliter.

(ii) *Preparation of sample solutions.* Dilute an accurately measured portion of the sample with sufficient distilled water to obtain a concentration of 0.02 milligram of loracarbef activity per milliliter (estimated).

(iii) *Procedure.* Using a suitable spectrophotometer and water as the blank, determine the absorbance of each standard and sample solution at the absorbance maximum at approximately 260 nanometers. Determine the exact position of the absorbance maximum for the particular instrument used.

(iv) *Calculations.* Determine the total amount of loracarbef dissolved as follows:

$$T = \frac{A_U \times c \times d \times 900}{A_s}$$

where:

T = Total milligrams of loracarbef activity dissolved;

A_U = Absorbance of sample;

A_S = Absorbance of the standard;

c = Concentration of the working standard solution in milligrams per milliliter; and

d = Dilution factor of the sample filtrate.

(17) *Cefadroxil hemihydrate.* Proceed as directed in paragraph (c)(1) of this section, except use the cefadroxil working standard and measure the absorbance at the absorption peak of approximately 264 nanometers.

(18) *Rifabutin*—(i) *Preparation of the working standard solution.* Accurately weigh approximately 45 milligrams of the rifabutin working standard into a suitable-sized volumetric flask. Dissolve and dilute to volume with 0.01*N* hydrochloric acid (prepared by diluting 5.0 milliliters of hydrochloric acid (37 percent) to 6 liters with distilled water) to obtain a concentration of approximately 13 micrograms rifabutin activity per milliliter.

(ii) *Preparation of sample solutions.* Forty-five minutes after the beginning of the rotation, withdraw a 10-milliliter aliquot from the vessel. Dilute a 2-milliliter portion of the sample to 25 milliliters with 0.01*N* hydrochloric acid.

(iii) *Procedure.* Using a suitable spectrophotometer and 0.01*N* hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance maximum at approximately 280 nanometers. Determine the exact position of the absorbance maximum for the particular instrument used.

(iv) *Calculations.* Determine the total amount of rifabutin dissolved as follows:

$$T = \frac{A_U \times c \times d \times 900}{A_s \times 1{,}000}$$

where:

T = Total milligrams of rifabutin activity dissolved;

A_U = Absorbance of sample;

A_S = Absorbance of the standard;

c = Rifabutin activity of the working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample filtrate.

(19) *Cefpodoxime proxetil*—(i) *Dissolution fluid: 0.04 molar glycine buffer, pH 3.0*—(A) *Stock solution.* Dissolve 54.5 grams of glycine (aminoacetic acid) and 42.6 grams of sodium chloride in about 500 milliliters of deionized water in a 1-liter volumetric flask. Add cautiously, and with swirling, 14.2 milliliters of concentrated hydrochloric acid. Cool to room temperature. Dilute to volume with deionized water and mix. Check the pH of the solution obtained by diluting 50 milliliters of the stock solution to 900 milliliters with deionized water. The pH should be 3.0±0.1. If necessary, adjust the pH of the stock solution with 50 percent sodium hydroxide or concentrated hydrochloric acid. Recheck that the pH of the working solution is 3.0±0.1.

(B) *Working solution.* Dilute 50 milliliters of stock solution to 900 milliliters with deionized water.

(ii) *Preparation of the working standard solutions.* Accurately weigh approximately 28 milligrams for the 100-milligram tablets and 56 milligrams for the 200-milligram tablets of the cefpodoxime proxetil working standard and dissolve in 10 milliliters of methanol. Dilute to 200 milliliters with dissolution fluid. Prepare fresh daily.

(iii) *Sample solutions.* Filter the sample solutions through a 0.45-micron filter before use. Use the sample solution as it is removed from the dissolution vessel without further dilution.

(iv) *Procedure.* Using a suitable spectrophotometer and water as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 259 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(v) *Calculations.* Determine the percent of label dissolved as follows:

$$\text{Percent dissolved} = (A_{sam}/A_{std}) \times (C_s/L) \times V \times P \times F1$$

where:

A_{sam} = Absorbance of the sample at 259 nanometers;

A_{std} = Absorbance of the working standard solution at 259 nanometers;

C_s = Concentration of the working standard preparation in milligrams per milliliter;

L = Tablet strength, in milligrams per tablet;

P = Purity of the reference standard in percent;

V = Volume of dissolution fluid used in milliliters (900); and

$F1$ = 0.7666 (conversion factor to free acid equivalents).

(d) *Evaluation.* Use the dissolution acceptance table and interpretation in the United States Pharmacopeia XXI.

[44 FR 48188, Aug. 17, 1979]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting § 430.215, see the List of CFR Sections Affected appearing in the Finding Aids section of this volume.

§ 436.216 High-performance liquid chromatographic assay.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard solution that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed

system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Trailing factor or asymmetry factor.* Calculate either the trailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline or the asymmetry factor (A_s) measured at a point 10 percent of the peak height from the baseline; whichever is required in the appropriate monograph, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:
$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

$$A_s = \frac{a+b}{2_a}$$

where:
a=Horizontal distance from point of ascent to point of maximum peak height; and
b=Horizontal distance from the point of maximum peak height to point of descent.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]$$

where:
n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
W_h=Peak width at half-height.
Calculate the absolute efficiency of the column, (reduced plate height) (h_r

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:
L=Length of column in centimeters;
n=Number of theoretical plates; and
d_p

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2\left(t_{Rj} - t_{Ri}\right)}{w_i + w_j}$$

where:
t_{Rj}=Retention time of a solute eluting after i (t_{Rj} is larger than $t_{Ri\geq}$)
t_{Ri}=Retention time for any solute;
w_i=Width of peak at baseline for any solute; and
w_j=Width of peak at baseline for any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left(\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right)^{1/2}$$

where:
$\overline{X}$ is the mean of N of individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

(5) *Capacity factor.* Calculate the capacity factor (k), if required in the monograph as follows:

$$k = \frac{t_r - t_m}{t_m}$$

where:
t_r=Retention time of solute; and
t_m=Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:
D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

[51 FR 11572, Apr. 4, 1986, as amended at 54 FR 47351, Nov. 14, 1989]

§ 436.217 Film-coat rupture test.

(a) *Immersion fluid.* Dilute 6.0 milliliters of hydrochloric acid to 1,000 milliliters with water. During the performance of the test maintain the immersion fluid at a temperature of 37±0.5 °C by using a thermostatically controlled water bath.

(b) *Immersion vessel.* Use a suitable vessel, such as a 1-liter beaker.

(c) *Operation.* Add 750 milliliters of immersion fluid to the immersion vessel.

(d) *Procedure.* Drop a tablet into the immersion fluid and record the time for the tablet coat to rupture. Repeat the test with a further 19 tablets, testing not more than 10 tablets with a given volume of immersion fluid.

(e) *Evaluation.* The tablets pass the film-coat rupture test if the mean coat rupture time does not exceed 20 seconds and not more than 2 tablets have a coat rupture time exceeding 40 seconds.

[52 FR 42432, Nov. 5, 1987]

Subpart F—Chemical Tests for Specific Antibiotics

§ 436.300 Polarimetric assay of carbenicillin indanyl sodium.

(a) *Equipment.* Polarimeter capable of measuring optical rotatory activity at 365 nanometers: Perkin-Elmer Model 141 or equivalent, with a suitable 1-decimeter polarimeter tube.

(b) *Reagents*—(1) *4-methyl-2-pentanone.* Meets ACS specifications.

(2) *Phosphate-citrate buffer.* Dissolve 61.0 grams of anhydrous disodium phosphate and 11.0 grams of citric acid in 950 milliliters of distilled water. Adjust the pH to 6.0 with 6*N* hydrochloric acid. Dilute to 1,000 milliliters with distilled water.

(c) *Preparation of carbenicillin indanyl sodium sample and working standard solutions.* Accurately weigh approximately 125 milligrams of the carbenicillin indanyl sodium sample or working standard into a 25-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer a 5-milliliter aliquot to a 50-milliliter glass-stoppered centrifuge tube. Add 15 milliliters of the phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper (4-methyl-2-pentanone solvent) phase and proceed as directed in paragraph (e) of this section.

(d) *Preparation of the blank.* Place a 5-milliliter aliquot of distilled water into a 50-milliliter glass-stoppered centrifuge tube, add 15 milliliters of phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone; stopper and shake the tube for 10 seconds. Centrifuge at 2,000 revolutions per minute for 10 minutes to separate the phases. Remove about 15 milliliters of the upper phase and proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Fill the polarimeter tube with the blank solution prepared as described in paragraph (d) of this section. Place the tube in the polarimeter. Adjust the polarimeter to zero rotation using a light source with a wavelength of 365 nanometers. Use the same procedure to determine the optical rotation of both the sample solution and the working standard solution prepared as directed in paragraph (c) of this section.

(f) *Calculations.* Calculate the carbenicillin content (potency) of the sample on an anhydrous basis as follows:

$$\text{Micrograms of carbenicillin per milligram of sample} = \frac{\text{Degrees of rotation of sample solution} \times \text{weight of working standard} \times 100 \times \text{micrograms of carbenicillin in each milligram of the working standard}}{\text{Degrees of rotation of working standard solution} \times \text{weight of sample} \times (100 - m)}$$

where: m=moisture content of the sample.

§ 436.301 Thin layer chromatography identity test for carbenicillin indanyl.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a), (b), (c), and (d) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches lined with Whatman's 3MM chromatographic paper (0.3 millimeters) or equivalent.

(2) *Iodine vapor chamber.* A rectangular tank approximately 9 × 9 × 3.5 inches, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 × 20 centimeters thin layer chromatography plates coated with silica gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Extraction solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 100:200:25:75:1.5 respectively.

(2) *Developing solvent.* Mix ethyl acetate, acetone, pyridine, water, and acetic acid in volumetric proportions of 300:400:25:75:2 respectively.

(3) *Ferric chloride-potassium ferricyanide reagent.* Immediately before use, mix 100 milliliters of a 1 percent ferric chloride solution in 1 percent hydrochloric acid with 100 milliliters of a 1 percent potassium ferricyanide solution and 75 milliliters of methanol.

(c) *Preparation of working standard solution.* Weigh an amount of the carbenicillin indanyl working standard equivalent to approximately 10 milligrams of carbenicillin into a 50-milliliter Erlenmeyer flask. Dissolve the material in sufficient extraction solvent to make a solution containing 1 milligram carbenicillin per milliliter.

(d) *Procedure.* Pour developing solvent into the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters of the standard solution and the sample solution. The plate should be air dried for 30 minutes. Place the plate into the chromatography tank. Allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Heat the plate for 30 minutes at 80° C. in a circulating air oven and then allow the plate to cool to room temperature. Place the plate in the iodine vapor chamber for about 30 seconds, remove the plate and spray it with the ferric chloride-potassium ferricyanide reagent. Carbenicillin indanyl appears as a blue spot on a yellow-green background at an R_f of about 0.5. The test is satisfactory if the sample compares qualitatively with the standard.

[39 FR 18944, May 30, 1974, as amended at 41 FR 18509, May 5, 1976]

§ 436.302 Clindamycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Barber-Colman 5,000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, dried over sodium sulfate.

(2) Chloroform, reagent grade.

(3) Acetic anhydride, reagent grade, used as acteylating agent.

(4) Internal standard: Prepare a solution containing 3 milligrams of cholestane per milliliter in pyridine.

(c) *Typical conditions.* (1) Column: 4 feet × 4 millimeters ID, glass, with 1 percent SE–30 on Diatoport S (60/80 mesh), or equivalent.

(2) Temperatures: Column 200° C.; detector 215° C.; injection port, ambient temperature.

(3) Carrier gas: Helium approximately 120 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen at 120 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 2 for clindamycin, 1 for internal standard: 2×10^{-8} amperes.

(d) *Preparation of clindamycin sample and working standard solutions.* Accurately weigh approximately 15 milligrams of sample or working standard into a glass-stoppered conical 15-milliliter centrifuge tube. Add 1.0 milliliter of chloroform, 1.0 milliliter of internal standard solution, and 0.6 milliliter of acetic anhydride. Agitate the tubes to insure dissolution of the sample and

complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10–15 minutes at 2,000–2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 0.5 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 60 percent deflection of the recorder scale by the clindamycin peak. The resolution of the peaks should be complete. The elution order is: Internal standard, clindamycin, and epiclindamycin (if present). Calculate the clindamycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard)/Area of internal standard peak;

R_s=Area of the clindamycin standard peak/Area of internal standard peak;

W_s=Weight of the clindamycin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of the clindamycin working standard in micrograms per milligram.

§ 436.303 Clindamycin content of clindamycin palmitate hydrochloride by vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Hewlett-Packard 7606[4] or equivalent.

(b) *Reagents.* (1) Acetic anhydride, reagent grade.

(2) Pyridine, reagent grade.

(3) Chloroform, reagent grade.

(4) Internal standard: Prepare a solution containing 5 milligrams of cholesteryl benzoate per milliliter in chloroform.

(c) *Typical conditions.* (1) Column: 6 feet × 2 millimeters ID, glass, with 1 percent UC–W98 on Chromosorb WHP (80/100 mesh) or equivalent.

(2) Temperatures: Column 275° C.; detector 290° C.; injection port 280° C.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame ionization—hydrogen at 12 pounds per square inch, air at 32 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 16; 1×10^{-9} amperes.

(d) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh approximately 15 milligrams of both the sample and the working standard into separate glass-stoppered, conical 15-milliliter centrifuge tubes. Add 1.0 milliliter of internal standard solution, 1.0 milliliter of pyridine, and 0.5 milliliter of acetic anhydride to each tube. Agitate the tubes to insure dissolution and complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10–15 minutes at 2,000–2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 1 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 40 percent deflection of the recorder scale by the clindamycin palmitate peak. The resolution of the peaks should be complete. The internal standard will be eluted before the clindamycin palmitate. Calculate the clindamycin content as directed in paragraph (f) of this section.

[4] Available from: Hewlett Packard Co., P.O. Box 301, Loveland, CO 80537.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard)/Area of internal standard peak;

R_s=Area of the clindamycin palmitate hydrochloride standard peak/Area of internal standard peak;

W_s=Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Micrograms of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

§ 436.304 Clindamycin phosphate vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector that has a sensitivity of at least 1×10^{-10} amperes: Hewlett-Packard 7600[4] or equivalent.

(b) *Reagents.* (1) Trifluoroacetic anhydride.

(2) Intestinal alkaline phosphatase.

(3) pH 9.0 borate buffer: Transfer 3.1 grams of boric acid into a 1-liter volumetric flask containing 500 milliliters of water, mix, and add 21 milliliters of 1.0*N* sodium hydroxide and 10 milliliters of 0.1*M* magnesium chloride. Dilute to volume with water and mix well.

(4) Internal standard: Prepare a chloroform solution containing approximately 0.45 milligram hexacosane per milliliter.

(5) Anhydrous sodium carbonate.

(c) *Typical conditions.* (1) Column: 2 feet × 3 millimeters ID, glass, with 1 percent SE–30 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column, 180° C., detector, 215° C., injection port, ambient temperature.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen flow at 40 milliliters per minute. Air flow at 400 milliliters per minute.

(5) Sensitivity: 1×10^{-9} amperes.

(d) *Preparation of clindamycin phosphate sample solution.* Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity[5] and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C.±2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool and proceed as directed in paragraph (f) of this section.

(e) *Preparation of the clindamycin hydrochloride standard solution.* Accurately weigh approximately 9 milligrams of the clindamycin hydrochloride working standard into a 35-milliliter glass-stoppered centrifuge tube and dissolve in 20 milliliters of pH 9.0 borate buffer. Proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Add 10 milliliters of the internal standard solution to each sample and standard solution. Shake the centrifuge tubes vigorously for 30 minutes and centrifuge. Remove the aqueous layer and discard. Shake the tubes again; mix in an ultrasonic mixer for 2 minutes, then centrifuge. No emulsion should be present at this stage. Remove the remaining aqueous layer by suction and transfer a 3-milliliter aliquot of the chloroform layer to a 1-dram tablet vial containing approximately 1 gram of anhydrous sodium sulfate. Swirl the vial to dry the chloroform and transfer a 1-milliliter aliquot to another 1-dram tablet vial. Using a 0.25-milliliter pipet, add 0.25 milliliter of trifluoracetic anhydride to

[4] See footnote 4 to § 436.303(a).

[5] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

each of the vials and place into a water bath at 45° C.±2° C. for 30 minutes. Remove the vials from the bath, add about 10 granules of anhydrous sodium carbonate to each vial, and allow to stand for approximately 30 minutes. Centrifuge the vials for approximately 10 minutes at 5,000 r.p.m. Inject 2 microliters of each of the resulting solutions into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The elution order is: Epiclindamycin (if present), clindamycin B (if present), clindamycin, and internal standard. Calculate the clindamycin content as directed in paragraph (g) of this section.

(g) *Calculations.* Calculate the clindamycin content of the sample as follows:

Micrograms of clindamycin per milligram=

$$\frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard)/Area of internal standard peak;

R_u=Area of the clindamycin standard peak/Area of internal standard peak;

W_s=Weight of the clindamycin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of the clindamycin working standard in micrograms per milligram.

[39 FR 18944, May 30, 1974, as amended at 41 FR 24704, June 18, 1976]

§ 436.305 Thin layer chromatographic identity test for hetacillin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix 650 milliliters acetone with 100 milliliters distilled water, 100 milliliters benzene, and 25 milliliters acetic acid.

(c) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters of ethanol.

(d) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solutions.* Prepare a solution containing 10 milligrams of an authentic hetacillin sample per milliliter in a 4:1 solution of acetone and 0.1*N* hydrochloric acid, and a solution of ampicillin standard at 1 mg/ml in the same solvent.

(e) *Procedure.* Spot a plate as follows: Apply approximately 10 microliters of the sample solution, 1 μ l, of the reference hetacillin solution, and 1 μ l, of the ampicillin reference solution on a line 1.5 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. Pour developing solvent into the glass trough in the bottom of the chromatography tank. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel about 11.5 centimeters from the bottom of the plate, remove the plate from the tank, and allow to air dry. Apply the spray solution (do not saturate) and place immediately into an oven maintained at 90° C. Heat 15 minutes.

(f) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[39 FR 18944, May 30, 1974, as amended at 45 FR 16472, Mar. 14, 1980]

§ 436.306 Lincomycin gas liquid chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector; Barber-Colman 5000 or equivalent.

(b) *Reagents.* (1) Pyridine, reagent grade, kept over potassium hydroxide.

(2) Methanol, reagent grade, anhydrous.

(3) Ethanol, absolute, reagent grade.

(4) Internal standard: Prepare a solution containing 2 milligrams of tetraphenylcyclopentadienone per milliliter in pyridine.

(5) Silylating reagent: Mix (9+1) of hexamethyldisilazane and trimethyl chlorosilane.

(c) *Typical conditions.* (1) Column: 4 feet × 3 millimeters ID, glass, with 3 percent SE–30 on Gas-Chrom Q (100/120 mesh), or equivalent.

(2) Temperatures: Column 225° C.; detector 280° C., injector 270° C.

(3) Carrier gas: Helium at 15 pounds per square inch.

(4) Detector: Hydrogen flame ionization—hydrogen at 20 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 100; attenuation 2; current 2×10^{-8} amperes.

(d) *Preparation of lincomycin sample and working standard solutions.* Prepare the sample and working standard as follows: Weigh accurately an aliquot of about 40 milligrams into a 10-milliliter volumetric flask, add sufficient pyridine to dissolve, and make to mark. Transfer a 1-milliliter aliquot to a glass-stoppered conical centrifuge tube and proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Add 0.2 milliliter of the silylating reagent to each centrifuge tube and allow to stand at least 30 minutes. Then add exactly 1 milliliter of the internal standard, shake well, and centrifuge. Inject 5 microliters of the supernatant into the gas chromatograph. Use the typical conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to provide at least 60 percent scale deflection with the lincomycin peak and to maintain a stable base line. The resolution of the peaks should be complete. The elution order is lincomycin B, lincomycin, and the internal standard. If necessary, adjust the current setting for the lincomycin B peak to give a satisfactory response relative to that of the lincomycin peak. Calculate the lincomycin content and lincomycin B content as directed in paragraph (f) of this section.

(f) *Calculations.*

$$\text{Lincomycin content of sample in micrograms per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of the lincomycin sample peak/Area of internal standard peak;

R_s=Area of the lincomycin standard peak/Area of internal standard peak;

W_s=Weight of the lincomycin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of lincomycin working standard in micrograms per milligram.

$$\text{Percent lincomycin B content} = \frac{B}{A+B} \times 100$$

where:

A=Area of lincomycin peak of the sample;

B=Area of lincomycin B peak of the sample corrected for the attenuation adjustment.

[39 FR 18944, May 30, 1974, as amended at 46 FR 3839, Jan. 16, 1981]

§ 436.307 Spectinomycin vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector; Barber-Colman 5,000 or equivalent.

(b) *Reagents.* (1) Dimethylformamide, reagent grade, kept dry over anhydrous sodium sulfate.

(2) Internal standard: Prepare a solution containing 2 milligrams of triphenylantimony per milliliter in dry dimethylformamide.

(3) Silylating reagent: Hexamethyldisilazane.

(c) *Typical conditions.* (1) Column: 4 feet by 4 millimeters ID, glass, with 5 percent SE–52 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column 215° C.; detector 270° C.; injection port 265° C.

(3) Carrier gas: Helium 93 milliliters per minute at 15 pounds per square inch.

(4) Detector: Hydrogen flame—hydrogen at 20 pounds per square inch, air at 40 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 10 for both spectinomycin and internal standard; 2×10^{-6} amperes.

(d) *Preparation of spectinomycin sample and working standard*—(1) *Working standard and bulk antibiotic solutions.* (i) Accurately weigh approximately 30 milligrams of sample or working standard into separate glass-stoppered 25-milliliter Erlenmeyer flasks.

(ii) Add 10 milliliters of the internal standard solution and 1.0 milliliter of hexamethyldisilazane to each flask. Agitate the flasks to insure dissolution of the sample and working standard

and complete mixing of the liquids. Shake the flasks intermittently for 1 hour. Proceed as directed in paragraph (e) of this section.

(2) *Finished product solutions.* Prepare the sample for assay as directed in the individual section for each antibiotic product to be tested.

(e) *Procedure.* Inject 2.5 microliters of each solution into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable base line and provide at least 60 percent deflection of the recorder scale by the spectinomycin peak. The resolution of the peaks should be complete. The internal standard will be eluted before spectinomycin. Calculate the spectinomycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the spectinomycin content of the sample as follows:

$$\begin{array}{c}\text{Micrograms}\\ \text{of spectinomycin}\\ \text{per milligram}\end{array} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard)/Area of internal standard peak;

R_s=Area of the spectinomycin standard peak/Area of internal standard peak;

W_s=Weight of the spectinomycin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of the spectinomycin working standard in micrograms per milligram.

§ 436.308 Paper chromatography identity test for tetracyclines.

(a) *Equipment*—(1) *Sheet* (*chromatographic*). Whatman No. 1 filter paper for chromatography, 20 × 20 centimeters.

(2) *Chamber* (*chromatographic*). Cylindrical glass chromatographic jar, 25 centimeters high by 12 centimeters in diameter, with a ground-glass lid.

(3) *Preparation of solutions*—(i) *pH3.5* buffer. Mix 13.93 volumes of 0.1*M* citric acid with 6.07 volumes of 0.2*M* of disodium phosphate.

(ii) *Solvent* (*organic phase*). Mix chloroform, nitromethane, and pyridine in volumetric proportions of 10:20:3, respectively.

(b) *Preparation of spotting solutions.* Prepare solutions of the working standard and sample as follows: Accurately weigh a portion of the working standard and sample and dilute with methanol to obtain a concentration of 1 milligram per milliliter of antibiotic to be tested.

(c) *Procedure.* Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Draw a starting line about 2.5 centimeters from and parallel to the bottom of the sheet. Wet the sheet thoroughly with the pH 3.5 buffer and blot it firmly between sheets of absorbent paper. Starting about 5 centimeters from the edge of the sheet and at 1.5-centimeter intervals, apply to the starting line 2 microliters each of standard solution, sample solution, and a 1:1 mixture of the standard and sample solutions. Allow a few minutes for the sheet to dry partially, and while still damp place it in the chamber with the bottom edge touching the solvent. When the solvent front has risen about 10 centimeters, remove the sheet from the chamber. Expose the paper to ammonia vapor. Examine the dried sheet under a strong source of ultraviolet light and record the position of any fluorescent spots. Measure the distance the solvent front traveled from the starting line and the distance that the fluorescent spots are from the starting line. Calculate the R_f value by dividing the latter by the former.

[39 FR 18944, May 20, 1974, as amended at 44 FR 30333, May 5, 1979; 45 FR 16472, 16474, Mar. 14, 1980]

§ 436.309 Anhydrotetracyclines and 4-epianhydrotetracycline.

Determination of 4-epianhydrotetracycline and anhydrotetracyclines in tetracycline, tetracycline hydrochloride, tetracycline phosphate, and in dosage forms thereof is as follows:

(a) *Screening procedure for total anhydrotetracyclines content*—(1) *Sample solution preparation*—(i) *Bulk packaged for repacking or for use in the manufacture of another drug.* Accurately weigh approximately 50 milligrams of the sample into a 50-milliliter volumetric flask and add 10 milliliters of 0.1*N* hydrochloric acid. Shake until sample is

completely dissolved, and then dilute to volume with water.

(ii) *Sterile dispensing containers.* Proceed as directed in paragraph (a)(1)(i) of this section.

(iii) *Capsules.* Transfer a representative quantity of capsule contents equivalent to 250 milligrams of tetracycline hydrochloride to a 250-milliliter volumetric flask. Add 50 milliliters of 0.1*N* hydrochloric acid and shake on a mechanical shaker for 5 minutes. Dilute to volume with water and filter through a fluted filter paper. Discard the first 20 milliliters of filtrate and collect the next 20 milliliters.

(iv) *Tablets.* Grind a representative number of tablets to a fine powder. Transfer an amount of the powder equivalent to 250 milligrams of tetracycline hydrochloride to a 250-milliliter volumetric flask. Add 50 milliliters of 0.1*N* hydrochloric acid and shake on a mechanical shaker for 5 minutes. Dilute to volume with water and filter through a fluted filter paper. Discard the first 20 milliliters of filtrate and collect the next 20 milliliters.

(v) *Oral powders and suspensions.* Proceed as described in paragraph (b) of this section.

(2) *Test procedure.* Using a suitable spectrophotometer, determine the absorbance of the sample solution prepared as directed in paragraph (a)(1) of this section at 430 millimicrons using 0.02*N* hydrochloric acid as a blank. Then accurately dilute 1.0 milliliter of the sample solution to 100 milliliters with 0.02*N* hydrochloric acid and determine the absorbance of this solution at 356 millimicrons, using 0.02*N* hydrochloric acid as a blank.

(3) *Calculations.*

$$\text{Percent anhydrotetracyclines} = \frac{[a430 - (a356 \times 0.0019)] \times 100}{195}$$

where:

*a*430=Absorptivity (1%, 1 cm.) of sample at 430 millimicrons;

$$\text{For bulk, absorptivity} = \frac{\text{Absorbance} \times 50 \times 10}{\text{Milligrams of sample}}$$

For sterile dispensing containers, capsules, and tablets; absorptivity=Absorbance×10; *a*356=Absorptivity (1%, 1 cm.) of sample at 356 millimicrons;

$$\text{For bulk, absorptivity} = \frac{\text{Absorbance} \times 50 \times 1000}{\text{Milligrams of sample}}$$

For sterile dispensing containers, capsules and tablets; absorptivity=Absorbance×1,000; 0.0019×Absorbance ratio (*A*430/*A*356) observed with tetracycline;

195=Absorptivity (1%, 1 cm.) of anhydrotetracycline hydrochloride at 430 millimicrons.

(4) *Evaluation.* If the total anhydrotetracyclines content determined by the screening procedure described in paragraph (a) of this section exceeds 2 percent for bulks and 3 percent for injectables, tablets, and capsules, perform the determination for anhydrotetracyclines and 4-epianhydrotetracycline described in paragraph (b) of this section. If the results of the test described in paragraph (a) of this section for total anhydrotetracyclines content are within the required limits in the case of bulks, injectables, tablets, and capsules, these results may be submitted in lieu of the results of the test for 4-epianhydrotetracycline and that test as described in paragraph (b) of this section need not be performed.

(b) *Determination of anhydrotetracyclines content and 4-epianhydrotetra-cycline content*—(1) *Apparatus and reagents*—(i) Chromatographic tubes (15 millimeters ID × 170 millimeters long having an outlet tube 4 millimeters ID × 50 millimeters long).

(ii) pH meter standardized at pH 7.0 and at pH 10.0.

(iii) Diatomaceous earth, acid-washed (Celite 545 or equivalent).

(iv) EDTA buffer. Dissolve 0.1 mole ethylenediaminetetraacetic acid disodium salt in 800 milliliters of water.

Adjust to pH 7.8 with ammonium hydroxide, reagent grade, and dilute to 1 liter with water.

(v) Chloroform, spectrophotometric grade.

(vi) Diluted ammonium hydroxide: Mix 1 volume of ammonium hydroxide, reagent grade, with 9 volumes of distilled water.

(vii) 0.1*N* hydrochloric acid.

(viii) 1.0*N* hydrochloric acid.

(2) *Preparation of support phase.* Add 5 milliliters of EDTA buffer to 10 grams of diatomaceous earth and mix until the diatomaceous earth is uniformly moistened. It will no longer be free-flowing.

(3) *Preparation of sample solutions.* Prepare the sample solutions as follows:

(i) *Tetracycline, tetracycline phosphate complex, and tetracycline hydrochloride bulk packaged for repacking or for use in the manufacture of another drug.* Place an amount of sample equivalent to 250 milligrams of tetracycline hydrochloride into a 50-milliliter beaker and dissolve in 10 milliliters of 0.1*N* hydrochloric acid. Immediately adjust the pH to 7.8 with the diluted ammonium hydroxide, and if necessary, with 1.0*N* hydrochloric acid and 0.1*N* hydrochloric acid. Quantitatively transfer this solution to a 50-milliliter volumetric flask by rinsing the beaker with EDTA buffer, fill to volume with EDTA buffer and shake well. Use this solution without delay to prepare a column as directed in paragraph (b)(4) of this section.

(ii) *Capsules.* Proceed as directed in paragraph (b)(3)(i) of this section, except pool the contents of a representative number of capsules and use an amount of the pooled capsule contents equivalent to 250 milligrams of tetracycline hydrochloride.

(iii) *Tablets.* Proceed as directed in paragraph (b)(3)(i) of this section, except grind tablets to a powder in a small mortar and use an amount of powder equivalent to 250 milligrams of tetracycline hydrochloride.

(iv) *Oral suspension and pediatric drops.* Place 5 milliliters of oral suspension equivalent to 125 milligrams of tetracycline hydrochloride or 2 milliliters of pediatric drops equivalent to 200 milligrams of tetracycline hydrochloride into a 50-milliliter beaker and add sufficient 0.1*N* hydrochloric acid to make 10 milliliters. Quickly adjust the pH to 7.8 with the diluted ammonium hydroxide, and if necessary, with 1*N* hydrochloric acid and 0.1*N* hydrochloric acid. Quantitatively transfer this solution to a 25-milliliter flask by rinsing the beaker with EDTA buffer, fill to volume with EDTA buffer, and shake well. Use this solution without delay to prepare a column as directed in paragraph (b)(4) of this section.

(v) *Oral powders.* Reconstitute as directed in the labeling and proceed as directed in paragraph (b)(3)(iv) of this section.

(vi) *Sterile dispensing containers.* Proceed as directed in paragraph (b)(3)(i) of this section.

(4) *Column preparation.* Pack support phase into the chromatographic tube by increments and firmly tamp down each increment. Do not use any glass wool in the column outlet. Add enough support phase to the column to reach a height of 9 to 11 centimeters; then add 1 milliliter of sample solution to 1 gram of diatomaceous earth in a small beaker, and mix thoroughly. Pack the sample: diatomaceous earth mixture on top of the column. Dry wash the beaker with support phase and pack an additional 1-centimeter layer of support phase on top of the sample layer.

(5) *Column elution and fraction collection.* Within 30 minutes after preparing the column, elute with chloroform. Collect 5 successive fractions of 5 milliliters, 5 milliliters, 10 milliliters, 10 milliliters, and 5 milliliters. During elution, two clear separate yellow bands will appear on the column. The first band is anhydrotetracyclines and will almost always elute in the first 5-milliliter fraction, but occasionally in the first and second 5-milliliter fractions. The second band is 4-epianhydrotetracycline and will elute in the remaining fractions. Label the fraction or fractions containing the first yellow band anhydrotetracyclines. Label the fractions after the first yellow band 4-epianhydrotetracycline. Determine the absorbance of each fraction at a wavelength of 438 nanometers using a suitable spectrophotometer equipped with a 1.0-centimeter cell and chloroform as the blank. If necessary,

make appropriate dilutions with choloroform to obtain a readable value.

(6) *Calculations*—(i) *Percent anhydrotetracyclines.* Calculate the percent anhydrotetracyclines as follows:

$$\text{Number of milligrams of anhydrotetracyclines in each fraction containing anhydrotetracyclines} = \frac{A \times b \times c}{20.28}$$

where:

A=Absorbance of the sample solution at 438 nanometers;

b=Volume of fraction in milliliters;

c=Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, *c* will be 5).

20.28=Absorptivity (1 milligram per milliliter, 1 centimeter) of anhydrotetracyclines in chloroform at 438 nanometers.

Total weight of anhydrotetracyclines in the sample=Sum of weights of anhydrotetracyclines in the fractions labeled anhydrotetracyclines × Number of milliliters in the sample solution

Percent anhydrotetracyclines in tetracycline, tetracycline hydrochloride, tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug=

$$100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Weight of the sample}}$$

Percent anhydrotetracyclines in dosage forms=

$$100 \times \frac{\text{Total weight of anhydrotetracyclines in the sample}}{\text{Tetracycline content of the sample}}$$

(ii) *Percent 4-epianhydrotetracycline.* Calculate the percent 4-epianhydrotetracycline as follows:

$$\text{Number of milligrams of 4-epianhydrotetracycline in each fraction labeled 4-epianhydrotetracycline} = \frac{A \times b \times c}{20.08}$$

where:

A=Absorbance of the sample solution at 438 nanometers;

b=Volume of the fraction in milliliters;

c=Dilution factor of the fraction (for example, if 2 milliliters of the fraction are diluted to 10 milliliters for reading, *c* will be 5);

20.08=Absorptivity (1 milligram per milliliter, 1 centimeter) of 4-epianhydrotetracycline in chloroform at 438 nanometers.

Total weight of 4-epianhydrotetracycline in the sample=Sum of weights of 4-epianhydrotetracycline in the fractions labeled 4-epianhydrotetracycline × Number of milliliters in the sample solution

Percent 4-epianhydrotetracycline in tetracycline, tetracycline hydrochloride, tetracycline phosphate complex bulk packaged for repacking or for use in the manufacture of another drug=

$$100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Weight of the sample}}$$

Percent 4-epianhydrotetracycline in dosage forms=

$$100 \times \frac{\text{Total weight of 4-epianhydrotetracycline in the sample}}{\text{Tetracycline content of the sample}}$$

[39 FR 18944, May 30, 1974, as amended at 40 FR 22251, May 22, 1975; 43 FR 11153, Mar. 17, 1978]

§ 436.310 Thin layer chromatography identity test for mitomycin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches, lined with filter paper and with a solvent trough on the bottom.

(2) *Plates.* Use 20 by 20 centimeter thin layer chromatography plates coated with silica gel G or equivalent, to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*-butanol, glacial acetic acid, and water in volumetric proportions of 4:2:1, respectively.

(2) *Spray solution.* Prepare a one-percent solution of ninhydrin in ethanol.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 1 milligram of mitomycin per milliliter, in water.

(d) *Procedure.* Pour the developing solvent into the solvent trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for 30 minutes. Prepare a plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 2 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 2 microliters of sample solution to points 2 and 3. After all spots are thoroughly dry, place the silica gel plate into the trough in the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel about 10 centimeters from the starting line. Remove the plate and allow it to air dry. After the plate is dry, spray lightly with the spray solution. Heat the plate in an oven at 110° C. for 10–15 minutes. Mitomycin appears as a pink spot.

(e) *Evaluation.* The sample and standard should have spots of corresponding R_f value (approximately 0.51), and standard and sample combined should appear as a single spot of corresponding R_f value.

[39 FR 18944, May 30, 1974, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.311 Thin layer chromatography identity test for amoxicillin.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a) through (e) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman's 3MM chromatographic paper (0.33 millimeters) or equivalent.

(2) *Plates.* Use 20- by 20-centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix methyl alcohol, chloroform, pyridine, and distilled water in volumetric proportions of 90:80:10:30, respectively.

(2) *Spray solution.* Dissolve 300 milligrams of ninhydrin in 100 milliliters of ethyl alcohol.

(c) *Preparation of working standard.* Weigh an amount of the amoxicillin working standard equivalent to 200 milligrams of amoxicillin into a 50-milliliter volumetric flask and bring to volume with 0.1*N* hydrochloric acid.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for at least 2 hours. Spot duplicate plates by applying approximately 5 microliters each of standard and sample solutions on a line 1.5 centimeters from the base of the plate and at intervals of not less than 2.0 centimeters. All solutions must be spotted within 10 minutes of preparation. Place spotted plate in a desiccator until solvent has evaporated from spots. Place the plate into the glass trough at the bottom of the chromatography tank. Cover the tank. Allow the solvent to reach the 15-centimeter scored mark, remove the plate from the tank and dry with a current of warm air until there is no detectable solvent odor. Apply the ninhydrin spray solution to the plate—do not saturate—and place immediately into an oven maintained at 110° C for 15 minutes.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. Amoxicillin has an R_f value of about 0.53. The sample and standard

should have spots of corresponding R_f values.

[39 FR 34032, Sept. 23, 1974; 48 FR 11427, Mar. 18, 1983, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.312 Atomic absorption method for determining the zinc content of zinc bacitracin.

(a) *Equipment.* An atomic absorbance spectrophotometer equipped with a zinc hollow-cathode discharge lamp, an air-acetylene flame, a nebulizer-burner system for introducing the sample solution into the flame, an optical dispersing device (such as a monochromator) for isolating a resonance line of zinc from others produced by the emission source, and a suitable radiation detector and recorder.

(b) *Preparation of working standard and sample solutions*—(1) *Workingstandard solutions.* Prepare a standard stock solution containing 10 milligrams of zinc per milliliter as follows: Weigh 3.11 grams of zinc oxide into a 250-milliliter volumetric flask, add 80 milliliters of 1*N* HCl, warm to dissolve, cool to room temperature, and dilute to volume with water. Dilute aliquots of this standard stock solution with 0.001*N* HCl to obtain three working standard solutions containing respectively 0.5, 1.5, and 2.5 micrograms of zinc per milliliter.

(2) *Sample solution.* Accurately weigh approximately 200 milligrams of the sample into a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.01*N* HCl. Transfer a 2.0-milliliter aliquot of this solution to a 200-milliliter volumetric flask and dilute to volume with 0.001*N* HCl.

(c) *Procedure.* Using 0.001*N* HCl as the blank, adjust the absorbance of the instrument to zero at a detection wavelength of 213.8 nanometers. Determine the absorbance of each standard solution and the sample solution at 213.8 nanometers.

(d) *Calculations.* Plot the absorbance versus the concentration of each of the working standard solutions. Draw a straight response line of best fit through these points. Read the concentration of zinc in micrograms per milliliter corresponding to the absorbance of the sample solution. Calculate the percent zinc in the sample as follows:

$$\text{Percent zinc} = \frac{C \times 100{,}000}{\text{Milligrams of sample} \times (100 - m)}$$

where:

C=Concentration of zinc in the sample solution in micrograms per milliliter;

m=Percent moisture in the sample.

[40 FR 15088, Apr. 4, 1975]

§ 436.316 Determination of penicillin G content.

(a) *Reagents.* The reagents are freshly prepared every three days and are of such quality that when used in this procedure with an authentic sample of penicillin G, not less than 97 percent of penicillin G is recovered.

(1) *Amyl acetate (iso-amyl acetate) solution.* Saturate the amyl acetate (boiling range 138.5° C—141.5° C) with the *N*-ethylpiperidine salt of penicillin G by adding 2 milligrams of the salt for each 1.0 milliliter of the solvent. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(2) *Acetone solution.* Saturate reagent grade acetone with the *N*-ethylpiperidine salt of penicillin G using 3 milligrams of salt for each 1 milliliter of acetone. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(3) *N-ethylpiperidine solution. N*-ethylpiperidine (boiling range 129.5° C—131.0° C) should be stored in brown bottles in a refrigerator. Dilute 1.0 milliliter of this reagent with 4.0 milliliters of amyl acetate. Saturate this solution with the *N*-ethylpiperidine salt of penicillin G, using about 3 milligrams of the salt for each 1.0 milliliter of solution. Cool this solution to 0° C—8° C and filter it through a sintered-glass filter immediately before use.

(4) *Phosphoric acid solution.* Prepare by dissolving 1.0 milliliter of reagent grade phosphoric acid (85 percent) in 4.0 milliliters of water. Cool to 0° C—8° C and shake before using.

(5) *Silica gel.* Use dry silica gel (mesh size 6–16, Tyler standard). Place about

0.5 gram of the silica gel in a micro filter funnel (approximately 10-millimeter diameter) having a fritted-glass disc of medium porosity.

(b) *Procedure.* Accurately weigh from 60 to 70 milligrams of the sample to be tested, except if penicillin G procaine is to be tested weigh 90 to 100 milligrams of sample, into a glass test tube or glass vial of approximately 10–milliliter capacity. Add 2.0 milliliters of water to dissolve or suspend (procaine) the penicillin and cool to 0° C—5° C. Add 2.0 milliliters of amyl acetate solution and 0.5 milliliter of phosphoric acid solution, stopper and shake the container vigorously for approximately 15 seconds. For penicillin G procaine, add a second 0.5-milliliter portion of phosphoric acid solution and shake vigorously. Centrifuge to obtain a clear separation of the two layers (approximately 20 seconds). If any penicillin procaine remains undissolved, add a third 0.5-milliliter portion of phosphoric acid solution, shake the container vigorously, and centrifuge. After centrifuging, remove as much of the amyl acetate layer as possible, usually about 1.7 milliliters to 1.8 milliliters, with a suitable hypodermic needle and syringe and place the portion removed into the filter funnel containing silica gel, described in paragraph (a)(5) of this section. Allow the amyl acetate to remain in contact with the silica gel for exactly 20 seconds, then apply suction and collect the filtrate in a small test tube placed in a suction flash surrounded by cracked ice. Pipet a 1.0-milliliter aliquot of the amyl acetate filtrate into a tared flat-bottom glass tube (approximately 15 x 50 millimeters) containing 1.0 milliliter of acetone solution and 0.5 milliliter of *N*-ethylpiperidine solution. The time elapsing between acidification and the addition of the filtrate to the above reagents should not be more than 3 minutes. Place the glass tube containing the mixture into a large weighing bottle, stopper the bottle and allow to stand for not less than 2 hours in a refrigerator at 0° C—8° C. Remove the liquid from the precipitate by means of a tared micro filter stick and wash with a total of 1.0 milliliter of acetone solution adding the latter by means of a hypodermic syringe equipped with a fine needle. Place the filter stick inside the glass tube, dry under vacuum at room temperature for not less than 1 hour, and weigh. (The *N*-ethylpiperidine penicillin G residues can be saved for saturating reagents).

(c) *Calculations.* Calculate the percent penicillin G content as follows:

$$\text{Percent penicillin G content} = \frac{\text{Milligrams } N\text{-ethylpiperidine penicillin precipitate} \times 149.4}{\text{Weight of sample in milligrams}}$$

[42 FR 59857, Nov. 22, 1977]

§ 436.317 Solubility characteristic test for griseofulvin (ultramicrosize) tablets.

(a) *Apparatus*—(1) *Vessel.* A cylindrical glass tank. The approximate dimensions are 40 centimeters in diameter and at least 23 centimeters in height.

(2) *Heating system.* A 1,500-watt immersion heating element connected to a partial immersion, contact thermometer and an appropriate control relay.

(3) *Circulating system components.* The circulating system consists of three different circulating devices:

(i) Circulating pump of a centrifugal, immersion type. Tubing approximately 1 centimeter outside diameter and 46 centimeters in length is attached to the pump outlet producing a flow rate of approximately 1,600 milliliters per minute when operated as described.

(ii) A ''4-element stirrer'' consisting of a motor and a shaft approximately 45 centimeters long and 8 millimeters in diameter. The motor rotates the vertical shaft in a clockwise direction at approximately 180 revolutions per minute. There are 4 elements or sets of stirring blades on the shaft. One set, located at the bottom of the shaft, is a 3-bladed element of 2.5 centimeters

overall radius with circular blades, 1.8 centimeters in diameter and 1 to 2 millimeters in thickness, pitched at an angle of approximately 45 degrees from the horizontal plane, so that fluid is propelled downward when the shaft is rotated in a clockwise direction. The three remaining sets of stirring blades have 4 blades each, symmetrically positioned about the shaft. Each set of blades is 3.2 centimeters in overall radius. Each blade is rectangular in shape, 2.4 centimeters in length, 1.2 centimeters in height, and 1 to 2 millimeters in thickness. The four sets of blades are located at 5 centimeter intervals on the shaft, the top three being fixed in a staggered configuration.

(iii) A rotating basket device consisting of a motor capable of constant speed of 100±5 revolutions per minute in a clockwise direction, a shaft, and a cylindrical basket. The shaft and the basket are fabricated from Type 316 stainless steel. The shaft is 6 millimeters in diameter and approximately 30 centimeters in length. It must run true on the motor axis so that the basket rotates smoothly and without perceptible wobble. The basket consists of two parts, one of which, the top, is attached to the shaft. It is of solid metal except for a 2-millimeter round vent, and is fitted with three spring clips that allow the removal of the lower part, or the basket proper, to admit the test sample. The detachable part of the basket is fabricated of welded seam stainless steel, 40 mesh woven wire cloth, formed into a cylinder 3.66 centimeters high and 2.5 centimeters in diameter, with a narrow rim of sheet metal around the top.

(4) *Circulating system configuration.* All three circulating devices are located in one half of the tank. In clockwise order they are the circulating pump, the rotating basket, and the 4-element stirrer. There is a distance of 12 to 13 centimeters between each of the three devices. The rotating basket shaft and the stirring shaft are located 9 to 10 centimeters from the tank wall. The 4-element stirrer is positioned 1 to 1.5 centimeters from the bottom of the tank. The rotating basket is fixed at 7 to 8 centimeters from the bottom. The circulating pump intake is located approximately 3 centimeters from the top of the fluid in the tank and 5 to 6 centimeters from the wall of the tank. The pump's outlet hose is held by a clamp so that hose makes a clockwise arc around the inside wall of the tank, descending to a point near the bottom of the tank and 5 to 6 centimeters from the wall, which is 180 degrees from the pump inlet.

(b) *Dissolution medium.* Distilled water.

(c) *Procedure.* Place 24 liters of dissolution medium into the vessel and maintain the temperature at 37±0.5° C by means of the heater, circulating pump, and the 4-element stirrer. Withdraw a 25-milliliter portion of the dissolution medium as a sample-blank solution. Place one tablet into the basket, and lower it into its proper position in the tank. Rotate the basket at 100±5 revolutions per minute in a clockwise direction. After 60 minutes, withdraw a second 25-milliliter portion as the sample solution. Filter the sample-blank solution and the sample solution through water-washed glass wool, or an equivalent filter, discarding the first 10 to 15 milliliters of each filtrate. Determine the amount of griseofulvin dissolved as directed in paragraph (d)(2) of this section.

(d) *Griseofulvin assay*—(1) *Preparation of standard solution and standard-blank solution.* Accurately weigh approximately 50 milligrams of griseofulvin working standard and place into a 100-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol. Transfer 2.0 milliliters of this solution to a 200-milliliter volumetric flask and dilute to volume with distilled water. This is the standard solution. Transfer a 2.0-milliliter portion of methyl alcohol to a 200-milliliter volumetric flask and dilute to volume with distilled water. This is the standard-blank solution. Filter the standard-blank solution and the standard solution through water-washed glass wool, or an equivalent filter, discarding the first 10 to 15 milliliters of each filtrate.

(2) *Procedure.* Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the four filtered solutions at the absorbance peak at approximately 295

nanometers, using suitable spectrophotometer cells with a 1-centimeter light path. Determine the exact position of the absorbance peak for the particular instrument used.

(3) *Calculation.* Determine the percentage of griseofulvin dissolved as follows:

$$\text{Percent griseofulvin dissolved} = \frac{A_u \times W_s \times V \times 10}{A_s \times P}$$

where:

A_u=Absorbance of the sample solution minus the absorbance of the sample-blank solution;

W_s=Weight of the working standard in milligrams;

V=Volume of the dissolution medium in liters;

A_s=Absorbance of the standard solution minus the absorbance of the standard-blank solution;

P=Labeled potency of the sample in milligrams of griseofulvin per tablet.

(e) *Evaluation.* The tablet passes the solubility characteristic test if it dissolves to the extent of not less than 50 percent at 60 minutes. If the tablet fails to meet this requirement, repeat the test on five additional tablets. The batch passes the solubility characteristic test if not less than 5 of 6 tablets meet the requirement.

[40 FR 41522, Sept. 8, 1975; 40 FR 45426, Oct. 2, 1975]

§ 436.318 Continuous flow thin layer chromatography identity test.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide equipped with a glass solvent trough in the bottom.

(2) *Plates.* Use a 20 × 20 centimeter thin-layer chromatography plate coated with Silica Gel G or equivalent to a thickness of 250 micrometers.

(3) *Cover.* A stainless steel cover with a slot, measuring 21 × 0.6 centimeters, cut in the front edge.

(4) *Supporting platform.* A platform that can be placed in the bottom of the chromatography tank so that the solvent trough is elevated about 3.75 centimeters.

(b) *Reagents*—(1) *Developing solvent.* Mix chloroform, redistilled methanol and concentrated ammonium hydroxide in volumetric proportions of 25:60:30, respectively.

(2) *Spray solution.* Dissolve 1 gram of ninhydrin in 100 milliliters of *n*- butanol and add 1 milliliter of pyridine.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 6 milligrams of antibiotic to be tested per milliliter in distilled water.

(d) *Procedure.* Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 1 centimeter, spot 3 microliters each of the standard solution and the sample solution. In addition, prepare one spot composed of 3 microliters of the sample solution and 3 microliters of the standard solution. Place the supporting platform in the bottom of the tank and place the solvent trough on it, near the front of the tank. Place a piece of Whatman #3 MM filter paper or equivalent, measuring 20x3 centimeters and folded in half, lengthwise, over the front edge of the tank to form a cushion and drying wick for the plate. Place the plate in the solvent trough with the coated side toward the front of the tank and leaning against the filter paper at the top. Pour the developing solvent into the trough and bottom of the tank. Cover the tank. The plate should extend approximately 1 centimeter beyond the top of the tank and through the slot in the cover. Seal all the openings in the tank with masking tape, except where the plate leans against the filter paper. Remove the plate from the tank after 5.5 hours. Allow the plate to air dry and then heat it for 15 minutes at 110° C in an oven. Remove the plate from the oven and immediately spray it with the spray solution. The compound appears as a pink spot.

(e) *Evaluation.* The sample and standard should have traveled the same distance from the origin, and the standard and sample combined should appear as a single spot that has traveled the same distance as the sample and standard individually.

[40 FR 57797, Dec. 12, 1975]

§ 436.319 Thin layer chromatography identity test for bacitracin and bacitracin zinc.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman 3MM chromatographic paper or equivalent.

(2) *Plates.* Use a 20- by 20-centimeter thin layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers. Activate the plate by heating for 20 minutes at 110° C. Allow to cool to room temperature and use immediately.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*- butanol, water, pyridine, glacial acetic acid, and ethyl alcohol in volumetric proportions of 60:10:6:15:5, respectively.

(2) *Spray solution.* Dissolve 1 gram of ninhydrin in a mixture of 1 milliliter of pyridine and sufficient *n*- butanol to make 100 milliliters.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 6.0 milligrams of bacitracin per milliliter in 1 percent disodium ethylenediamine tetraacetic acid in water.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for at least 30 minutes. Prepare a plate as follows: On a line 2.0 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot approximately 1.0 microliter of the standard solution to points 1 and 3. When these spots are dry, apply approximately 1.0 microliter of sample solution to points 2 and 3. After all spots are thoroughly dry, place the base of the silica gel plate directly into the glass trough in the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 13 centimeters from the starting line. Remove the plate from the tank, and allow it to air dry. After the plate is dry, spray lightly with the spray solution. The plate may take 1 hour or more to develop at room temperature. The development may be speeded up by warming the plate in a 110° C oven.

(e) *Evaluation.* The sample and standard should have spots of corresponding R_f value (approximately 0.26) and standard and sample combined should appear as a single spot of corresponding R_f value.

[42 FR 27228, May 27, 1977]

§ 436.320 Ferric chloride colorimetric assay.

(a) *Reagents.* (1) 1*N* hydrochloric acid.

(2) 0.01*N* hydrochloric acid.

(3) Ferric chloride stock solution. Quickly weigh (very hygroscopic) 5.0 grams of $FeCl_3 \cdot 6H_2O$ into a 100-milliliter beaker. Add approximately 10 milliliters of 1*N* hydrochloric acid and stir to dissolve. Quantitatively transfer to a 50-milliliter glass-stoppered amber volumetric flask and make up to volume with water.

(4) Ferric chloride working reagent. Pipette 10.0 milliliters of ferric chloride stock solution into a 2-liter volumetric flask, add 20 milliliters 1*N* hydrochloric acid, and bring to volume with water. Check the pH; it should be between 2.0 and 2.1.

(b) *Standard solution.* Accurately weigh approximately 50 milligrams of the working standard of the antibiotic to be tested and dissolve with 25 milliliters of 0.1*N* hydrochloric acid. Quantitatively transfer to a 250-milliliter volumetric flask and dilute to volume with distilled water. Keep in a glass-stoppered flask and store under refrigeration. Discard solution after 7 days.

(c) *Sample solution.* Accurately weigh approximately 50 milligrams of the sample and dissolve with 25 milliliters of 0.1*N* hydrochloric acid. Quantitatively transfer to a 250-milliliter volumetric flask and dilute to volume with distilled water.

(d) *Procedure.* Pipette exactly 10.0 milliliters of the standard solution and of the sample solution into separate test tubes. To each tube add exactly 10 milliliters of ferric chloride working reagent, mix, and allow to stand 15 minutes. Determine the absorbance of each solution at 490 nanometers in a suitable spectrophotometer against a blank prepared from 10.0 milliliters of

0.01*N* hydrochloric acid and 10.0 milliliters of ferric chloride working reagent.

(e) *Estimation of potency.* Calculate the potency as follows:

$$\text{Micrograms of antibiotic per milligram} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \text{Potency of standards in micrograms per milligram}$$

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978]

§ 436.321 Griseofulvin gas liquid chromatography.

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector: Hewlett Packard 7600 or equivalent.

(b) *Reagents.* (1) Chloroform, reagent grade.

(2) Internal standard solution: Prepare a solution containing 1.0 milligram of tetraphenylcyclopentadienone per milliliter in chloroform.

(c) *Typical conditions*—(1) *Column.* 1.2 meters by 4 millimeters ID, glass, packed with 1 percent OV–17 on Gas Chrom Q (100/120 mesh), or equivalent.

(2) *Temperatures.* Column 245° C; detector 260° C; injection port 260° C.

(3) *Carrier gas.* Helium approximately 60 millimeters per minute and 40 pounds per square inch (1.7 kilograms per square centimeter).

(4) *Detector.* Hydrogen flame ionization-hydrogen at 12 pounds per square inch (0.5 kilogram per square centimeter), air at 34 pounds per square inch (1.43 kilograms per square centimeter).

(5) *Sensitivity.* Adjusted to obtain peak heights greater than 50 percent full scale deflection.

(d) *Preparation of griseofulvin sample and working standard solutions.* Accurately weigh approximately 40 milligrams of both the sample and the working standard into separate 25-milliliter volumetric flasks. Add sufficient internal standard solution to dissolve the contents of each flask with vigorous mixing and then dilute to volume with internal standard solution and mix. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Inject 1.0 microliter of this solution into the gas chromatograph. Use the typical conditions and materials listed in paragraphs (a), (b), and (c) of this section. The resolution of the peaks should be complete. The griseofulvin peak will elute before the internal standard peak. Calculate the griseofulvin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the griseofulvin content of the sample as follows:

$$\text{Micrograms of griseofulvin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

W_u=Weight of the sample in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram.

[44 FR 20660, Apr. 6, 1979]

§ 436.322 High-pressure liquid chromatographic assay for anthracycline antibiotics.

(a) *Equipment.* A suitable high-pressure liquid chromatograph, such as a Waters Associates Model 244[1] or equivalent equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

[1] Available from Waters Associates, Inc., Maple St., Milford, Mass. 10757.

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator;

(6) A 30-centimeter column having an inside diameter of 4.6 millimeters and packed with a suitable reverse phase packing such as: Waters Associates, Micro-Bondapak C18.[1]

(b) *Reagents.* (1) Solvent mixture: Water: acetonitrile (69:31).

(2) Mobile phase: Water: acetonitrile (69:31) adjusted to pH 2 with phosphoric acid. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(3) Internal standard solution: Prepare a 2.0-milligram-per-milliliter solution of 2-naphthalenesulfonic acid in the solvent mixture.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Procedure.* Use the standard and sample solutions prepared as directed in the individual monographs for the drug being tested. Use the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section. Inject 5 microliters of the standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components (ordinarily this time is 20 minutes). After separation of the standard solution has been completed, inject 5 microliters of the sample solution into the chromatograph and repeat the procedure described for the standard solution. The elution order is: Void volume, internal standard, doxorubicin, dihydrodaunomycin, daunomycin, adriamycinone, dihydrodaunomycinone, bromodaunomycin, daunomycinone, and bis-anhydrodaunomycinone.

(e) *Calculations.* Calculate the anthracycline content as directed in the individual monograph for the drug being tested.

[43 FR 44836, Dec. 29, 1978]

§ 436.323 Continuous flow thin layer chromatography identity test for cefamandole nafate.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide equipped with a glass solvent trough in the bottom.

(2) *Plates.* Use a 20 x 20 centimeter thin-layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers.

(3) *Cover.* A stainless steel cover with a slot measuring 21 x 0.6 centimeters, cut in the front edge.

(4) *Supporting platform.* A platform that can be placed in the bottom of the chromatography tank so that the solvent trough is elevated about 3.75 centimeters.

(b) *Reagents*—(1) *Developing solvent.* Mix *n*- butanol, glacial acetic acid, and water in volumetric proportions of 4:1:1, respectively.

(2) *Spray solution.* Mix starch iodide solution, glacial acetic acid, and 0.1 *N* iodine test solution, U.S.P. in volumetric proportions of 50:3:1. Prepare the starch iodide solution by mixing starch iodide paste test solution, U.S.P. and water in volumetric proportions of 1:1.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 1 milligram of cefamandole nafate per milliliter in distilled water.

(d) *Procedure.* Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 1 centimeter, spot 5 microliters each of the standard solution and the sample solution. In addition, prepare one spot composed of 5 microliters of the sample solution and 5 microliters of the standard solution. Place the supporting platform in the bottom of the tank and place the solvent trough on it, near the front of the tank. Place a piece of Whatman #3 MM filter paper or equivalent, measuring 20 x 3 centimeters and folded in half, lengthwise, over the front edge of the tank to form a cushion and drying

wick for the plate. Place the plate in the solvent trough with the coated side toward the front of the tank and leaning against the filter paper at the top. Pour the developing solvent into the trough and bottom of the tank. Cover the tank. The plate should extend approximately 1 centimeter beyond the top of the tank and through the slot in the cover. Seal all the openings in the tank with masking tape, except where the plate leans against the filter paper. Remove the plate from the tank after 4 hours. Allow the plate to air dry and then heat it in an oven for 15 minutes at 110° C. Remove the plate from the oven and immediately spray it with the spray solution. The compound appears as a white spot on a purple background.

(e) *Evaluation.* The sample and standard should have traveled the same distance from the origin, and the combined standard and sample should appear as a single spot that has traveled the same distance as the sample and standard individually.

[44 FR 20664, Apr. 6, 1979]

§ 436.324 Polarographic analysis of cefamandole.

(a) *Equipment*—(1) *Polarograph.* Use a polarograph equipped with a dropping mercury indicating electrode, a platinum auxilliary electrode, and a saturated calomel reference electrode, such as Princeton Applied Research Model 174[1] or equivalent.

(2) *X–Y plotter.* Use a suitable X–Y plotter, such as Houston Omnigraphic Model 2200–3–3[2] or equivalent.

(3) *Nitrogen.* Use a nitrogen tank equipped with a pressure-reducing regulator and a filter to remove traces of oxygen, such as an oxisorb filter[1] or equivalent.

(b) *Reagent.* pH 2.3 Buffer: Dissolve 3.6 grams of dibasic sodium phosphate, 39.4 grams of citric acid, and 70.8 grams of potassium chloride in sufficient distilled water to make 1 liter.

(c) *Operating conditions*—(1) Operating mode: Differential pulse.

(2) Scan range: −0.3 volt to −1.05 volts.

(3) Scan rate: −2 millivolts per second.

(4) Sensitivity: 10 to 20 microamperes or equivalent to keep peak on scale.

(5) Mercury drop time: 1 second per drop.

(6) Modulation amplitude: 25 millivolts.

(7) Display direction: +

(8) Damping: None.

(d) *Preparation of sample and working standard solutions.* Use the cefamandole lithium working standard. Accurately weigh approximately 12 milligrams of sample or working standard into a 50-milliliter volumetric flask. Dissolve the sample or working standard in 4 milliliters of distilled water. Immediately prior to polarography, add 30 milliliters of pH 2.3 buffer, dilute to volume with distilled water, and mix.

(e) *Procedure.* Transfer a portion of the sample or working standard solution to the polarographic cell. Pass a stream of nitrogen through the solution for 5 minutes to remove the dissolved oxygen. After 5 minutes, disperse the nitrogen above the sample. Start the mercury dropping from the mercury dropping electrode, and, using the operating conditions described in paragraph (c) of this section, record the polarogram. Compare the polarogram of the sample to that of the working standard.

(f) *Calculations.* Calculate the potency of cefamandole as follows:

$$\text{Micrograms of cefamandole per milligram} = \frac{A \times \text{Milligrams of working standard} \times \text{Potency of working standard in micrograms per milligram}}{B \times \text{Milligrams of sample}}$$

where:

A=The peak height of the sample;

[1] Available from Princeton Applied Research Corporation, P.O. Box 2565, Princeton, NJ 08540.

[2] Available from Houston Instrument, 8500 Cameron Road, Austin, TX 78753.

B=The peak height of the working standard.

The peak height is obtained from the polarogram by measuring the vertical distance from the peak to the baseline of the sample or working standard.

[44 FR 20664, Apr. 6, 1979, as amended at 47 FR 20756, May 14, 1982]

§ 436.325 High pressure liquid chromatography assay for vidarabine.

(a) *Equipment.* A suitable high pressure liquid chromatograph, such as a Waters Associates Model 244[1] or equivalent, equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A 30-centimeter column having an inside diameter of 4 millimeters and packed with a suitable octadecyl bonded silica phase packing such as Waters Associates, Micro-Bondapak C18.[1]

(b) *Mobile phase.* (1) Transfer 2.2 grams of sodium dioctyl sulfosuccinate and 10 milliliters of glacial acetic acid to a 1-liter volumetric flask. Dissolve with 500 milliliters of methanol, dilute to volume with distilled water, and mix. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter.

(2) De-gas the mobile phase just before its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh approximately 24 milligrams of sample or working standard into a 200-milliliter volumetric flask. Add about 150 milliliters of distilled water and heat on a steam bath for 10 minutes. Shake until all the powder is dissolved. Cool to room temperature and dilute to volume with distilled water.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the sample or working standard solution prepared as directed in paragraph (d) of this section into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. The elution order is void volume, 9-β-D-arabinofuranosylhypoxanthine (if present), vidarabine, and adenine (if present).

(f) *Calculations.* Calculate the vidarabine content as follows:

$$\text{Micrograms of vidarabine per milligram} = \frac{A \times W_s \times f}{B \times W_u}$$

where:

A=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);

B= Area of the standard peak;

W_s=Weight of standard in milligrams;

W_u=Weight of sample in milligrams; and

f=Potency of standard in micrograms per milligram.

[44 FR 30334, May 25, 1979, as amended at 47 FR 23708, June 1, 1982]

§ 436.326 Thin layer chromatographic identity test for cefoxitin sodium.

Using the sample solution prepared as described in the section for the antibiotic drug to be tested, proceed as described in paragraphs (a), (b), (c), (d), and (e) of this section.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman #3 MM, chromatographic paper or equivalent.

(2) *Plates.* Use a 20×20 centimeter thin layer chromatography plate coated with silica gel G or equivalent to a thickness of 250 micrometers.

(b) *Developing solvent.* Mix ethyl acetate, pyridine, *n*-butanol, acetic acid,

[1] Available from Waters Associates, Inc., Maple St., Milford, MA 10757.

and water in volumetric proportions of 42:21:21:6:10, respectively.

(c) *Spray solution.* Immediately before use, mix 100 milliliters of a 1-percent ferric chloride solution in 1 percent hydrochloric acid with 100 milliliters of a 1-percent potassium ferricyanide solution and 75 milliliters of methanol.

(d) *Preparation of working standard solution.* Prepare a solution containing approximately 2.5 milligrams per milliliter of cefoxitin working standard in distilled water.

(e) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters each of the standard solution and the sample solution. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough. Cover and seal the tank. Allow the solvent front to travel about 15 centimeters from the starting line. Remove the plate from the tank and heat it for 1 hour at 60° C in a circulating air oven. Remove the plate from the oven and allow it to cool at room temperature. Apply the spray solution and allow it to air dry. After approximately 15 minutes, the compound appears as a blue spot on a yellow-green background.

(f) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[44 FR 10373, Feb. 20, 1979, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.327 Thin layer chromatographic identity test for cyclacillin.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 x 23 x 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* One percent ammonium formate aqueous solution.

(2) *Spray solution.* Dilute starch iodide paste TS (U.S.P. XIX) with an equal volume of water. Mix diluted starch iodide paste, glacial acetic acid, and 0.1*N* iodine in volumetric proportions of 50:3:1, respectively.

(c) *Assay solutions*—(1) *Preparation of working standard solution.* Accurately weigh an amount of cyclacillin working standard and dissolve the material with sufficient 0.1*N* sodium hydroxide to obtain a solution containing 1 milligram per milliliter. Allow the solution to stand for 15 minutes before using.

(2) *Preparation of sample solution.* Using the sample solution prepared as described in the section for the antibiotic to be tested, proceed as described in paragraphs (d) and (e) of this section.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank. Cover and seal the tank. Allow it to equilibrate. Prepare a plate as follows: On a line 2 centimeters from the base of the thin layer chromatography plate and at intervals of 2 centimeters, spot 5 microliters each of the working standard solution and sample solution. Dry the spots thoroughly with a stream of dry air. Place the plate in the trough in the chromatography tank. Cover and seal the tank. Allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Dry the plate by heating for 30 minutes at 80° C in a circulating air oven. Visualize the spots by applying the spray solution.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the spots are from the starting line. Divide the latter by the former to calculate the R_f value. The sample and standard should appear as white spots against a blue background at an R_f of approximately 0.6. The test is satisfactory if the R_f value of the sample compares with that of the working standard.

[46 FR 2981, Jan. 13, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.328 High pressure liquid chromatographic assay for sulfisoxazole acetyl content.

(a) *Equipment.* A suitable high pressure liquid chromatograph, such as a Waters Associates Model 244[1] or equivalent equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with a suitable reverse phase packing such as: Waters Associates, Micro-Bondapak C18;[1] and

(6) A suitable integrator.

(b) *Reagents*—(1) *Mobile phase.* Mix acetonitrile (high pressure liquid chromatography grade): water (40:60). Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. De-gas the mobile phase just prior to its introduction into the chromatograph pumping system.

(2) *Internal standard solution.* Dissolve 0.33 milligram of benzanilide per milliliter in acetonitrile (high pressure liquid chromatography grade). Filter the solution through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the baseline.

(d) *Preparation of the working standard and sample solutions*—(1) *Working standard solution.* Prepare a solution containing 1.0 milligram per milliliter of sulfisoxazole acetyl in the internal standard solution.

(2) *Sample solution.* Reconstitute the sample as directed in the labeling. Allow to stand for 1 hour. Shake gently and transfer 5.0 milliliters of the sample to a separatory funnel. Extract the suspension three times with 75-milliliter portions of chloroform. Collect the chloroform layers in a 250-milliliter volumetric flask. Dilute the flask to volume with chloroform and mix. Filter a portion of the solution through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. Transfer a 4.0-milliliter aliquot of the filtrate into a 25-milliliter glass-stoppered flask and evaporate to dryness under a stream of dry air. Dissolve the residue in 10.0 milliliters of the internal standard solution, stopper, and mix.

(e) *Procedure.* Using the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 5 microliters of sample or working standard solution prepared as described in paragraph (d) of this section, into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. The elution order is void volume, sulfisoxazole acetyl and benzanilide.

(f) *Calculations.* Calculate the sulfisoxazole content as follows:

$$\text{Milligrams of sulfisoxazole per milliliter of sample} = \frac{A \times \text{Concentration of the standard solution in milligrams per milliliter} \times 125 \times 0.864}{B}$$

where:

A=Area of sample peak (at a retention time equal to that of the standard) divided by the area of the internal standard peak;

B=Area of the standard peak divided by the area of the internal standard peak;

[1] Available from: Waters Associates, Inc., Maple Street, Milford, MA 10757.

0.864=The molecular weight of sulfisoxazole divided by the molecular weight of sulfisoxazole acetyl.

[46 FR 2990, Jan. 13, 1981]

§ 436.329 High-pressure liquid chromatographic assay for meclocycline.

(a) *Equipment.* A suitable high-pressure liquid chromatograph, such as a Waters Associates Model 244[1] or equivalent equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 340 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator;

(6) A column approximately 25 centimeters in length having an inside diameter of approximately 4 millimeters and packed with a suitable reverse-phase packing such as: 10 micrometer silica gel particles bonded to octadecyl silane, Vydac 201 TP Reverse Phase[2] or equivalent.

(b) *Reagents*—(1) *0.001M Ammonium (ethylenedinitrilo) tetraacetate.* Moisten 293 milligrams of (ethylenedinitrilo) tetraacetic acid with 1 milliliter of methanol and dissolve in 7 milliliters of concentrated ammonium hydroxide. Dilute to 900 milliliters with distilled water, adjust the pH to 6.6 with glacial acetic acid, and dilute to 1,000 milliliters with distilled water.

(2) *Mobile phase.* Mix 150 milliliters of tetrahydrofuran (high-pressure liquid chromatography grade) with 850 milliliters of 0.001*M* ammonium (ethylenedinitrilo) tetraacetate. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 0.8 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh an amount of sample or working standard equivalent to approximately 25 milligrams of meclocycline into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methanol and mix. Transfer exactly 3.0 milliliters of this solution to a 25-milliliter volumetric flask, dilute to volume with mobile phase, and mix.

(e) *Procedure.* Using the equipment, reagents, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the sample or working standard solution prepared as described in paragraph (d) of this section into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. The elution order is void volume, oxytetracycline (if present), demeclocycline (if present), methacycline (if present), and meclocycline.

(f) *Calculations.* Calculate the meclocycline content as follows:

$$\text{Micrograms of meclocycline per milligram} = \frac{A \times \text{Milligrams of working standard} \times \text{Potency of the working standard in micrograms per milligram}}{B \times \text{Milligrams of sample}}$$

where:

A= Area or peak height of the sample peak (at a retention time equal to that observed for the standard);

B= Area or peak height of the standard peak.

[46 FR 3836, Jan. 16, 1981]

[1] Available from: Waters Associates, Inc., Maple St., Milford, MA 10757.

[2] Available from: The Separations Group, 16640 Spruce St., Hesperia, CA 92345.

§ 436.330 Thin layer chromatographic identity test for bacampicillin.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 × 23 × 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover, lined with Whatman's 3MM chromatographic paper (0.3 millimeter) or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel 60F 254 or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix methylene chloride, chloroform, and 95 percent ethyl alcohol in volumetric proportions of 100:10:10, respectively.

(2) *Spray solution.* Dissolve 1 gram of ninhydrin in 100 milliliters of *n*-butanol and add 1 milliliter of pyridine.

(c) *Spotting solutions*—(1) *Preparation of working standard solution.* Dissolve and dilute a weighed amount of the bacampicillin hydrochloride working standard with sufficient 95 percent ethyl alcohol to obtain a solution containing 2 milligrams per milliliter.

(2) *Preparation of sample solution.* Dissolve and dilute a weighed amount of the sample with sufficient 95 percent ethyl alcohol to obtain a solution containing 2 milligrams per milliliter. Proceed as described in paragraphs (d) and (e) of this section.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for one hour. Prepare a plate as follows: On a line 2.5 centimeters from the base of the thin layer chromatography plate and at intervals of 2.0 centimeters, spot 5 microliters of the working standard solution to positions 1 and 3. When these spots are dry, apply 5 microliters of the sample solution to points 2 and 3. After all the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank, allow the solvent front to travel about 15 centimeters from the starting line (about 30 minutes) and then remove the plate from the tank. Air dry the plate. Visualize the spots by spraying with spray solution and heating in an oven at 100° C for approximately 10 minutes.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the spots are from the starting line. Divide the latter by the former to calculate the R_f value. Bacampicillin appears as a purple spot at an R_f value of approximately 0.52. The test is satisfactory if the R_f value of the sample compares with that of the working standard. The combined spot should appear as a single spot of corresponding R_f value.

[46 FR 25602, May 8, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.331 High-pressure liquid chromatographic assay for dactinomycin.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Mobile phase.* Mix acetonitrile (high-pressure liquid chromatography grade): water (60:40). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of*

working standard solution. Prepare a solution containing 0.25 milligram per milliliter of dactinomycin in mobile phase.

(2) *Preparation of sample solution.* Prepare the sample solution as described in the individual monograph for the drug being tested.

(e) *Procedure.* Use the equipment, mobile phase, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by two or more replicate injections of 10 microliters each of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and, calculate the relative standard deviation as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the minimum performance requirement for the relative standard deviation is not more than 1.0 percent. If the minimum performance requirement is not met, adjustment must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the dactinomycin content as described in the individual monograph for the drug being tested.

[49 FR 24017, June 11, 1984, as amended at 50 FR 5749, Feb. 12, 1985]

§ 436.332 High-pressure liquid chromatographic assay for moxalactam.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, U.S.P. XX;

(5) A suitable recorder of at least 25.4 centimeter deflection;

(6) A suitable integrator.

(b) *Mobile phase.* Mix 0.01*M* ammonium acetate:methanol (19:1). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 0.5 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Transfer the contents of an ampoule of working standard to a tared weighing bottle. Place the unstoppered weighing bottle in a desiccator containing a saturated aqueous solution of potassium carbonate to provide an atmosphere of 42 percent relative humidity. Allow the moisture content of the working standard to equilibrate for 16 hours. Determine the moisture content as described in § 436.201 of this chapter. Equilibrated standard material must be kept in a closed weighing bottle and used within 36 hours of equilibration. Dissolve approximately 50 milligrams of the working standard, accurately weighed and corrected for moisture, with sufficient distilled water to obtain a solution containing 0.5 milligram of

moxalactam per milliliter. Use the prepared solution immediately.

(e) *Preparation of sample solution.* Mix contents of vial thoroughly. Dissolve an accurately weighed portion of approximately 50 milligrams of sample with distilled water to obtain a concentration of 0.5 milligram per milliliter (estimated); also, reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with distilled water to obtain a concentration of 0.5 milligram per milliliter (estimated). Use the prepared solution immediately.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 5 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 5 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(g) *Calculations.* (1) Calculate the moxalactam content in micrograms per milligram as follows:

$$\text{Micrograms of moxalactam per milligram of sample} = \frac{R_u \times W_s \times P}{R_s \times W_u}$$

where:

R_u=Sum of the areas of the moxalactam sample R-isomer and the S-isomer peaks;

R_s=Sum of the areas of the moxalactam working standard R-isomer and the S-isomer peaks;

W_u=Weight of the sample in milligrams;

W_s=Weight of the moxalactam working standard in milligrams;

P=Potency of the moxalactam working standard in micrograms per milligram, corrected for moisture.

(2) Calculate the moxalactam content of the vial as follows:

$$\text{Milligrams of moxalactam per vial} = \frac{R_u \times W_s \times P \times \mathrm{d}}{R_s \times 100{,}000}$$

where:

R_u=Sum of the areas of the moxalactam R-isomer and the S-isomer peaks;

R_s=Sum of the areas of the moxalactam working standard R-isomer and the S-isomer peaks;

W_s=Weight of the moxalactam working standard in milligrams;

P=Potency of the moxalactam working standard in micrograms per milligram, corrected for moisture;

d=Dilution factor.

(3) Calculate the ratio of R-isomer to S-isomer as follows:

$$\text{Ratio of R-isomer to S-isomer} = \frac{\text{Area of the R-isomer peak}}{\text{Area of the S-isomer peak}}$$

[46 FR 61069, Dec. 15, 1981]

§ 436.333 Thin layer chromatographic identity test for moxalactam.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top. Line the inside walls of the tank with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use a 20x20 centimeter thin layer chromotagraphy plate coated with silca gel G or equivalent to a thickness of 250 micrometers.

(b) *Developing solvent.* Mix ethyl acetate, glacial acetic acid, acetonitrile, and water in volumetric proportions of 42:14:14:18, respectively.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 10 milligrams per milliliter of moxalactam in distilled water.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the silica gel plate, and at intervals of 2 centimeters, spot 10 microliters each of the standard solution and the sample solution. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough. Cover and seal the tank. Allow the solvent front to travel about 15 centimeters from the starting line. Remove the plate from the tank and air dry. Expose the plate to iodine vapors for 40 minutes. Immediately circumscribe all spots using a suitable marker.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values and intensity.

[46 FR 61070, Dec. 15, 1981, as amended at 49 FR 2242, Jan. 19, 1984]

§ 436.334 High-pressure liquid chromatographic assay for piperacillin.

(a) *Equipment.* A high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator;

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter (United States Pharmacopeia XX).

(b) *Reagents.* (1) 0.2*M* monobasic sodium phosphate: Dissolve 27.60 grams of monobasic sodium phosphate with sufficient water to make 1,000 milliliters.

(2) 10 percent tetrabutylammonium hydroxide in water.

(3) Ampicillin-piperacillin solution: Dissolve and dilute 25 milligrams of ampicillin and 5 milligrams of piperacillin monohydrate with sufficient mobile phase to obtain 100 milliliters, and mix.

(c) *Mobile phase.* Methanol:water:0.2*M* monobasic sodium phosphate:10 percent tetrabutylammonium hydroxide (450 : 447 : 100:3) adjusted to pH 5.5±0.02 with phosphoric acid. The concentration of reagents may be varied to obtain acceptable operation of the system. De-gas the mobile phase just prior to its introduction into the chromatograph pumping system.

(d) *Preparation of working standard and sample solutions*—(1) *Working standard solution.* Place approximately 20 milligrams of the working standard, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(2) *Sample solution*—(i) *Micrograms per milligram.* Place approximately 20 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Add 25 to 30 milliliters of mobile phase. Shake until dissolved. Dilute to volume with mobile phase.

(ii) *Milligrams per vial.* Reconstitute as directed in the labeling. Withdraw the total contents and dilute with mobile phase to a concentration of 0.4 milligram of piperacillin per milliliter.

(e) *Procedure.* Use the equipment, reagents, mobile phase, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section and proceed as directed in paragraph (e) of this section.

(1) *Systems suitability test.* Chromatograph three replicate samples of ampicillin-piperacillin solution as directed in paragraph (e)(2) of this section. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the resolution factor as described for system suitability tests in the United States Pharmacopeia XX General Chapter 621 for gas chromatography. The resolution factor between ampicillin and piperacillin is not

less than 15. If the resolution factor does not meet this limit, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Operate the high-pressure liquid chromatograph at ambient temperature at a flow rate of one milliliter per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. Purge the column with mobile phase until a steady baseline is established. Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations*—(1) Calculate the piperacillin content in micrograms per milligram as follows:

$$\text{Micrograms of piperacillin per milligram of sample} = \frac{A \times \text{Weight of standard in milligrams} \times \text{Potency of working standard in micrograms per milligram}}{B \times \text{Weight of sample in milligrams}}$$

where:

A=Area of the sample peak (at a retention time equal to that observed for the standard);

B=Area of the standard peak.

(2) Calculate the piperacillin content in grams per vial as follows:

$$\text{Grams of piperacillin per vial} = \frac{A \times \text{Milligrams of standard in milligrams per milliliter} \times \text{Potency of working standard in micrograms per milligram} \times d}{B \times 1{,}000 \times 1{,}000}$$

where:

A=Area of the sample peak (at a retention time equal to that observed for the standard);

B=Area of the standard peak;

d=Dilution factor.

[47 FR 15768, Apr. 13, 1982; 47 FR 33493, Aug. 3, 1982]

§ 436.335 High-pressure liquid chromatographic assay for chloramphenicol palmitate.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 280 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, U.S.P. XX.

(b) *Mobile phase.* Mix methanol:water:glacial acetic acid (170:30:1). Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the reference standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of sample and working standard solutions.* Accurately weigh approximately 65 milligrams of sample

or chloramphenicol palmitate working standard each into a 50-milliliter volumetric flask. Add approximately 35 milliliters of methanol and 1 milliliter of glacial acetic acid. Place in an ultrasonic bath for 10 minutes and dilute to volume with methanol.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the chloramphenicol content as follows:

$$\begin{array}{c}\text{Micrograms of}\\ \text{chloramphenicol}\\ \text{per milligram}\end{array} = \frac{(A)(W_s)(f)}{(B)(W_u)}$$

where:

A=Area of chloramphenicol palmitate sample peak (at a retention time equal to that observed for the standard);

B=Area of the working standard peak;

W_s=Weight of standard in milligrams;

W_u=Weight of sample in milligrams; and

f=Micrograms of chloramphenicol activity per milligram of chloramphenicol palmitate working standard.

[49 FR 6091, Feb. 17, 1984]

§ 436.336 Thin layer chromatographic identity test for azlocillin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top.

(2) *Iodine vapor chamber.* A rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Buffer.* Dissolve 9.078 grams of potassium phosphate, monobasic (KH_2PO_4) in sufficient distilled water to make 1,000 milliliters (solution A). Dissolve 17.88 grams of sodium phosphate, dibasic, heptahydrate ($Na_2HPO_4.7H_2O$) in sufficient distilled water to make 1,000 milliliters (solution B). Place 12.1 milliliters of solution B into a 100-milliliter volumetric flask and dilute to volume with solution A.

(2) *Developing solvent.* Place 50 milliliters of *n*-butyl acetate, 9 milliliters of *n*-butanol, 25 milliliters of glacial acetic acid, and 15 milliliters of buffer into a separatory funnel. Shake well and allow the layers to separate. Discard the lower phase and use the upper phase as the developing solvent.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 20 milligrams of azlocillin per milliliter in distilled water.

(d) *Procedure.* Pour developing solvent into the glass trough on the bottom of the chromatography tank to a depth of about 1 centimeter. Use the chamber immediately. Prepare plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 10 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 10 microliters of sample solution to points 2 and 3. Place spotted plate in a desiccator until solvent has evaporated from spots. Place the plate into the glass trough at the bottom of the chromatography tank. Cover the tank. Allow the solvent to travel about 15 centimeters from the starting line. Remove the plate from the tank and allow to air dry. Warm the iodine vapor chamber to vaporize the iodine crystals and place the dry plate in the iodine vapor chamber until the spots are visible, usually about 10 minutes.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The azlocillin sample and the standard should have spots of corresponding R_f values (approximately 0.4), and standard and sample combined should appear as a single spot for

azlocillin. The penicilloate and penilloate of azlocillin as well as ampicillin appear as additional spots with R_f values of approximately 0.15, 0.3, and 0.25, reectively.

[47 FR 53348, Nov. 26, 1982]

§ 436.337 High-pressure liquid chromatographic assay for cephradine.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 8 millimeters;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder that is compatible with the detector output;

(5) A suitable integrator (optional); and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents.* (1) 4 percent glacial acetic acid.

(2) 3.86 percent sodium acetate.

(c) *Mobile phase.* 4 percent glacial acetic acid:3.86 percent sodium acetate:methanol:distilled water (3:15:200:782). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase prior to its introduction into the chromatograph pumping system. The distilled water:methanol ratio may be varied to obtain acceptable operation of the system.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the cephradine in the cephradine working standard that is about 75 percent of full scale.

(e) *Preparation of working standard and sample solutions*—(1) *Preparation of cephradine working standard solution.* Place an accurately weighed portion of the cephradine working standard into a suitably sized container. Add 5.0 milliliters of distilled water and place in an ultrasonic bath to facilitate dissolution. Dilute with a sufficient amount of mobile phase to obtain a solution containing 0.8 milligram of cephradine activity per milliliter.

(2) *Preparation of cephalexin working standard solution.* Dissolve an accurately weighed portion of the cephalexin working standard with mobile phase to obtain a solution containing 0.02 milligram of cephalexin activity per milliliter. Place in an untrasonic bath to facilitate dissolution.

(3) *Preparation of sample solutions*—(i) *Product not packaged for dispensing (micrograms of cephradine per milligram).* Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(ii) *Product packaged for dispensing.* Determine both micrograms of cephradine per milligram of the sample and milligrams of cephradine per container. Use separate containers for preparation of each sample solution as described in paragraphs (e)(3)(ii) (*a*) and (*b*) of this section.

(*a*) *Micrograms of cephradine per milligram.* Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(*b*) *Milligrams of cephradine per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Using this sample solution,

proceed as directed in paragraph (f)(1) of this section.

(f) *Procedure*—(1) *Cephradine content.* Using the equipment, reagents, mobile phase, and operating conditions as listed in paragraphs (a), (b), (c), and (d) of this section, inject 10 microliters of the cephradine working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(3)(i) of this section into the chromatograph and repeat the procedure described for the working standard solution. The elution order is void volume, cephalexin, and cephradine. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(3)(ii) (*a*) and (*b*) of this section.

(2) *Cephalexin content.* Proceed as directed in paragraph (f)(1) of this section, except:

(i) Use a detector sensitivity setting that gives a peak height for the cephalexin in the cephalexin working standard that is about 75 percent of full scale; and

(ii) Use the cephalexin working standard in lieu of the cephradine working standard.

(g) *Calculations.* (1) Calculate the micrograms of cephradine per milligram of sample as follows:

$$\text{Micrograms of cefoperazone per milligram milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cephradine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cephradine peak in the chromatogram of the cephradine working standard;

P_s=Cephradine activity in the cephradine working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m= Percent moisture content of the sample.

(2) Calculate the cephradine content of the vial as follows:

$$\text{Milligrams of cefoperazone per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cephradine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the chephradine peak in the Chromatogram of the cephradine working standard;

P_s=Cephradine activity in the cephradine working standard solution in micrograms per milliliter;

C_s=Milligrams of the standard per milliliter; and

d=Dilution factor of the sample.

(3) Calculate the percent cephalexin content of the sample as follows:

$$\text{Percent cephalexin} = \frac{A_a \times W_b \times P_b \times 10}{A_b \times W_u \times (100 - m)}$$

where:

A_a=Area of the cephalexin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_b=Area of the cephalexin peak in the chromatogram of the cephalexin working standard;

W_b=Milligrams of cephalexin per milliliter of cephalexin working standard solution;

W_u=Milligrams of cephradine per milliliter of sample solution;

P_b=Micrograms of cephalexin per milligram of cephalexin working standard; and

m=Percent moisture content of the sample.

[49 FR 47483, Dec. 5, 1984]

§ 436.338 High-pressure liquid chromatographic assay for cefoperazone.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator;

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and

packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, United States Pharmacopeia XX.

(b) *Mobile phase.* Mix 1.2 milliliters 1*M* triethylammonium acetate, 2.8 milliliters 1*M* acetic acid, and 120 milliliters acetonitrile in a one liter flask and dilute to volume with distilled water. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatographic pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Dissolve approximately 40 milligrams of working standard, accurately weighed, with mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter.

(e) *Preparation of sample solutions*—(1) *Product not packaged for dispensing (micrograms of cefoperazone per milligram).* Dissolve an accurately weighed portion of the sample with sufficient mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(2) *Product packaged for dispensing.* Determine both micrograms of cefoperazone per milligram of the sample and milligrams of cefoperazone per container. Use separate containers for preparation of each sample solution as described in paragraph (e)(2)(i) and (ii) of this section.

(i) *Micrograms of cefoperazone per milligram.* Dissolve and accurately weighed portion of the sample with sufficient mobile phase to obtain a solution containing 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(ii) *Milligrams of cefoperazone per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute and aliquot of this solution with mobile phase to a concentration of 0.16 milligram of cefoperazone activity per milliliter. Using this sample solution, proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(1) of this section into the chromatograph and repeat the procedure described for the working standard solution. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(2)(i) and (ii) of this section.

(g) *Calculations*—(1) Calculate the micrograms of cefoperazone per milligram of sample as follows:

$$\text{Micrograms of cefoperazone per milligram milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefoperazone sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the cefoperazone working standard peak;

P_s=Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m= Percent moisture content of the sample.

(2) Calculate the cefoperazone content of the vial as follows:

$$\text{Milligrams of cefoperazone per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefoperazone sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the cefoperazone working standard peak;

P_s=Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

[48 FR 789, Jan. 7, 1983; 48 FR 7439, Feb. 22, 1983; 48 FR 28250, June 21, 1983]

§ 436.339 High-pressure liquid chromatographic assay for bleomycin fractions.

(a) *Equipment.* A high-pressure liquid chromatograph equipped with:

(1) Two solvent pumps;

(2) A solvent programmer;

(3) A low dead volume cell 8 to 20 microliters;

(4) A light path length of 1 centimeter;

(5) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(6) A suitable recorder;

(7) A suitable integrator; and

(8) A suitable-sized column approximately 25 centimeters in length having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *0.005M 1-pentanesulfonic acid in 0.5 percent acetic acid adjusted to pH 4.3 with concentrated ammonium hydroxide.* Filter and degas before using.

(2) *Methanol, spectrophotometric grade.* Filter and degas before using.

(3) *Mobile phase.* Adjust the solvent programmer for linear gradient development starting with a mixture of 0.005*M* 1-pentanesulfonic acid:methanol (9:1) and ending with a mixture of 0.005*M* 1-pentanesulfonic acid:methanol (6:4) in 1 hour at a flow rate of 1.2 milliliters per minute. Minor flow rate and gradient changes can be made as necessary depending on column and instrument conditions. Disodium ethylenediaminetetraacetic acid USP at a concentration of 0.005*M* may be added to the mobile phase if necessary for satisfactory performance.

(c) *Preparation of sample solution.* Reconstitute the vial with 6 milliliters of deaerated water.

(d) *Procedure.* Using the equipment and reagents listed in paragraphs (a) and (b) of this section, start pumping the mobile solvent at the initial conditions. Inject 10 microliters of the sample solution into the chromatograph and begin the linear gradient pumping program. After the final mobile phase conditions are reached (1 hour) continue to pump the solvent mixture for an additional 20 minutes or until the demethylbleomycin A_2 is eluted. The elution order is void volume, bleomycinic acid, bleomycin A_2, bleomycin A_5, bleomycin B_2, bleomycin B_4, and demethylbleomycin A_2.

(e) *Calculations.* Calculate the percentage of each bleomycin by comparing its peak area contribution to that of the total response of all the bleomycins.

[48 FR 51912, Nov. 15, 1983]

§ 436.340 High-pressure liquid chromatographic assay for tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles.

(b) *Mobile phase.* Dissolve 0.55 gram of monobasic ammonium phosphate in 900 milliliters of water. Adjust the pH to 1.8 with concentrated phosphoric acid and dilute to 1 liter with water. Mix 800 milliliters of this solution with 200 milliliters of methanol. Filter the mobile

phase through a suitable glass fiber filter that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatography pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the 4-epitetracycline peak that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Working standard solution.* Accurately weigh approximately 18 milligrams of the tetracycline hydrochloride working standard into a 50-milliliter volumetric flask. Into the same flask, accurately weigh approximately 38 milligrams of the 4-epitetracycline working standard. Dissolve and dilute to volume with a methanol:water mixture (7:18).

(2) *Sample solution.* Reconstitute the sample as directed in the labeling. Transfer 10.0 milliliters of the reconstituted sample into a 50-milliliter volumetric flask and dilute to volume with a methanol:water mixture (7:18).

(e) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution. The elution order is 4-epitetracycline followed by tetracycline.

(f) *Calculations.* Calculate the tetracycline hydrochloride and 4-epitetracycline hydrochloride content as follows:

$$\text{Milligrams of tetracycline hydrochloride per milliliter of sample} = \frac{A_1[(W_1 \times B) + (W_e \times C)]}{A_2 \times 1{,}000}$$

$$\text{Milligrams of 4-epitetracycline hydrochloride per milliliter of sample} = \frac{A_3[(W_e \times D) + (W_t \times E)]}{A_4 \times 1{,}000}$$

where:

A_1=Area of the tetracycline sample peak (at a retention time equal to that observed for tetracycline in the tetracycline working standard);

A_2=Area of the tetracycline peak in the tetracycline working standard;

A_3=Area of the 4-epitetracycline sample peak (at a retention time equal to that observed for the 4-epitetracycline peak in the 4-epitetracycline working standard);

A_4=Area of the 4-epitetracycline peak in the 4-epitetracycline working standard;

W_t=Milligrams of the tetracycline working standard;

W_e=Milligrams of the 4-epitetracycline working standard;

B=Percent tetracycline hydrochloride in the tetracycline working standard;

C=Percent tetracycline hydrochloride in the 4-epitetracycline working standard;

D=Percent 4-epitetracycline hydrochloride in the 4-epitetracycline working standard; and

E=Percent 4-epitetracycline hydrochloride in the tetracycline working standard.

[48 FR 51290, Nov. 8, 1983]

§ 436.341 High-pressure liquid chromatographic assay for plicamycin.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 280 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 0.01*M* phosphoric acid.

(2) *Mobile phase.* Mix acetonitrile (high-pressure liquid chromatography grade):0.01*M* phosphoric acid (350:650). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution.* Place approximately 5 milligrams of the plicamycin working standard, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(2) *Preparation of sample solution.* Prepare the sample solution as described in the individual monograph for the drug being tested.

(e) *Procedure.* Use the equipment, reagents, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by two or more replicate injections of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the relative standard deviation as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the minimum performance requirement for the relative standard deviation is not more than 2.0 percent. If the minimum performance requirement is not met, adjustment must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the plicamycin content as described in the individual monograph for the drug being tested.

[49 FR 24017, June 11, 1984, as amended at 50 FR 5749, Feb. 12, 1985]

§ 436.342 High-pressure liquid chromatographic assay for cefazolin.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection; and

(5) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *Buffer solution, pH 3.6.* Transfer 0.9 gram of sodium phosphate, dibasic USP and 1.298 grams of citric acid USP to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(2) *Buffer solution, pH 7.0.* Transfer 5.68 grams of sodium phosphate, dibasic

USP and 3.63 grams of potassium phosphate monobasic to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water and mix.

(3) *Mobile phase.* Mix buffer solution, pH 3.6: acetonitrile (9:1). Filter through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(4) *Internal standard solution.* Transfer 1.2 grams of salicylic acid to a 200-milliliter volumetric flask. Dissolve in 10 milliliters of methyl alcohol, dilute to volume with buffer solution, pH 7.0, and mix.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the initial baseline.

(d) *Preparation of working standard and sample solutions*—(1) *Working standard solution.* Place approximately 50 milligrams of cefazolin working standard, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(2) *Sample solution.* Place approximately 50 milligrams of the sample, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with buffer solution, pH 7.0, and mix. Transfer 4.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution prepared as directed in paragraph (d)(1) of this section into the chromatograph. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (d)(2) of this section into the chromatograph and repeat the procedure described for the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components. The elution order is void volume, salicylic acid and cefazolin.

(f) *Calculation.* Calculate the micrograms of cefazolin per milligram of sample as follows:

$$\text{Micrograms of cefazolin per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u = Area of the cefazolin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard) /Area of internal standard peak;

R_s = Area of the cefazolin peak in the chromatogram of the cefazolin working standard/Area of internal standard peak;

P_s = Cefazolin activity in the cefazolin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

[48 FR 33478, July 22, 1983; 48 FR 34947, Aug. 2, 1983]

§ 436.343 High-pressure liquid chromatographic assay for cefuroxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 15-centimeter column having an inside diameter of 4.6 millimeters and packed with hexyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers in diameter.

(b) *Reagents*—(1) *Acetate buffer, pH 3.4.* Place 50 milliliters of 0.1*M* sodium acetate into a 1,000-milliliter volumetric flask and dilute to volume with 0.1*M* acetic acid. Mix.

(2) *Mobile phase.* Mix 0.1*M* acetate buffer, pH 3.4:acetonitrile (10:1). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(3) *Internal standard solution.* Prepare a 1.5 milligram per milliliter solution of orcinol monohydrate in water.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the cefuroxime working standard with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours.

(2) *Preparation of sample solutions*—(i) *Product not packaged for dispensing (micrograms of cefuroxime per milligram).* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(ii) *Product packaged for dispensing.* Determine both micrograms of cefuroxime per milligram of the sample and milligrams of cefuroxime per container. Use separate containers for preparation of each sample solution as described in paragraphs (d)(2)(ii) (*a*) and (*b*) of this section.

(*a*) *Micrograms of cefuroxime per milligram.* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution containing 1.0 milligram of cefuroxime per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(*b*) *Milligrams of cefuroxime per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with distilled water to obtain a stock solution of 1.0 milligram per milliliter. Immediately transfer 5.0 milliliters of the stock solution to a 100-milliliter volumetric flask, add 20.0 milliliters of internal standard solution and dilute to 100 milliliters with distilled water and mix. Store the solution in a refrigerator and use within 6 hours. Using this sample solution, proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (d)(2)(i) of this section into the chromatograph and repeat the procedure described for the working standard solution. If the sample is packaged for dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (d)(2)(ii)(*a*) and (d)(2)(ii)(*b*) of this section.

(f) *Calculations.* (1) Calculate the micrograms of cefuroxime per milligram of sample as follows:

$$\text{Micrograms of cefuroxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) Calculate the cefuroxime content of the vial as follows:

$$\text{Milligrams of cefuroxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter; and

d= Dilution factor of the sample.

[48 FR 38460, Aug. 24, 1983; 48 FR 40704, Sept. 9, 1983]

§ 436.344 Thin layer chromatographic identity test for cefuroxime.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel F or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform, methanol, and formic acid in volumetric proportions of 90:16:4, respectively.

(c) *Preparation of the spotting solutions.* Dissolve approximately 200 milligrams each of the working standard and sample in 5 milliliters of a 50 percent aqueous acetone solution.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot 5 microliters each of the sample and working standard solutions. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel a minimum of 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry. Observe under ultraviolet light (254 nanometers).

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[48 FR 38461, Aug. 24, 1983]

§ 436.345 High-pressure liquid chromatographic assay for ceftizoxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *pH 3.6 buffer solution.* Transfer 2.31 grams of sodium phosphate diabasic dodecahydrate and 1.42 grams of citric acid monohydrate to a

1-liter volumetric flask. Dissolve and dilute to volume with distilled water.

(2) *pH 7.0 buffer solution.* Transfer 14.33 grams of sodium phosphate dibasic dodecahydrate and 3.63 grams of potassium phosphate monobasic to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water.

(3) *Mobile phase.* Mix pH 3.6 buffer solution:acetonitrile (9:1). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(4) *Internal standard solution.* Place 1.2 grams of salicyclic acid in a 200-milliliter volumetric flask. Dissolve in 10 milliliters of methyl alcohol, dilute to volume with pH 7.0 buffer solution and mix.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the ceftizoxime working standard with sufficient pH 7.0 buffer solution to obtain a solution containing 1,000 micrograms of ceftizoxime activity per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix.

(e) *Preparation of sample solutions*—(1) *Product not packaged for dispensing (micrograms of ceftizoxime per milligram).* Dissolve an accurately weighed portion of the sample with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(2) *Product packaged for dispensing.* Determine both micrograms of ceftizoxime per milligram of the sample and milligrams of ceftizoxime per container. Use separate containers for preparation of each sample solution as described in paragraphs (e)(2) (i) and (ii) of this section.

(i) *Micrograms of ceftizoxime per milligram.* Dissolve an accurately weighed portion of the sample with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram of ceftizoxime per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(ii) *Milligrams of ceftizoxime per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency is a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of the solution thus obtained with sufficient pH 7.0 buffer solution to obtain a concentration of 1.0 milligram per milliliter. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution and mix. Using this sample solution, proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Using the equipment, reagents, and operating conditions as listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. The elution order is void volume, ceftizoxime, and internal standard. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution prepared as described in paragraph (e)(1) of this section into the chromatograph and repeat the procedure described for the working standard solution. If the sample is packaged for

dispensing, repeat the procedure for each sample solution prepared as described in paragraphs (e)(2) (i) and (ii) of this section.

(g) *Calculations*—(1) Calculate the micrograms of ceftizoxime per milligram of sample as follows:

$$\text{Micrograms of ceftizoxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the ceftizoxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s=Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

$$\text{Milligrams of ceftizoxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the ceftizoxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of internal standard peak;

P_s=Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

[48 FR 46270, Oct. 12, 1983; 48 FR 49656, Oct. 27, 1983]

§ 436.346 High-pressure liquid chromatographic assay for cyclosporine.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 4,500 pounds per square inch and a flow rate of at least 5 milliliters per minute;

(2) A suitable ultraviolet detection system operating at a wavelength of 210 nanometers;

(3) A suitable recorder;

(4) A suitable integrator;

(5) An oven or water bath capable of maintaining the column at an operating temperature of 70° C;

(6) A steel capillary tube, 1 meter in length, having an inside diameter of 0.25 millimeter. This tube is inserted between the injection system and the chromatographic column and is equilibrated to 70° C; and

(7) A sample injection valve on which the loop determines the sample size.

(b) *Columns.* The chromatographic column is packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials that exhibit some degree of polarity such as the hydrocarbon bonded silicas with dimethyl, trimethyl, or octyl groups. Connect a saturation column gravity packed with similarly bonded silica particles 40 to 60 microns in diameter to the inlet of the analytical column.

(c) *Mobile phase.* Mix acetonitrile, water, methanol, and *o*-phosphoric acid (900:525:75:0.075 by volume). Degas by passing through a 0.5-micrometer filter with vacuum and ultrasonicate for no less than 2 minutes before use. The mobile phase may be sparged perceptibly with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of acetonitrile to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(d) *Operating conditions.* Perform the assay at a constant operating temperature of 70° C with a typical flow rate of 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with a typical chart speed of 2.5 millimeters per minute. Obtain chromatograms for performance parameters at a chart speed of not less than 25 millimeters per minute to allow a more accurate measurement of peak geometry.

(e) *Preparation of working standard and sample solutions.* Prepare the working standard and sample solutions as directed in the individual monographs for cyclosporine.

(f) *Systems suitability.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by about 5 injections of not less than 10 microliters each of working standard solution. Proceed

with the analysis when the following minimum performance requirements have been met or exceeded.

(1) *Capacity ratio factor.* Calculate the capacity ratio (k) of the cyclosporine peak as follows:

$$k = \frac{t - t_m}{t_m}$$

where:

t=Retention time of solute; and

t_m=Retention time of solvent or unretained substance.

The capacity ratio is satisfactory if it is not less than 3 or not more than 10.

(2) *Coefficient of variation.* The coefficient of variation of at least five replicate injections is less than 1 percent.

(3) *Efficiency.* Calculate the efficiency (n) as follows:

$$n = 5.545\left(\frac{t}{W_{0.5}}\right)^2$$

where:

t=Retention time of solute; and

$W_{0.5}$=Peak width at half height. Both t and $W_{0.5}$ must be measured in the same units.

The efficiency is satisfactory if it is greater than 1,500 theoretical plates when assaying cyclosporine and greater than 700 theoretical plates when assaying finished dosage forms.

(4) *Asymmetry factor.* Calculate the asymmetry factor (A_s) as follows:

$$A_s = \frac{W_{0.1}}{2f}$$

where:

$W_{0.1}$=Horizontal distance measured from a point on the cyclosporine peak ascent 10 percent above the baseline to an intercept with the cyclosporine peak descent; and

f=Horizontal distance from point of 10 percent ascent above the baseline of the cyclosporine peak to point of maximum peak height.

The asymmetry factor is satisfactory if it is not more than 1.5.

(5) *Resolution.* Calculate the resolution (R_s) as follows:

$$R_s = \frac{2\,(t_j - t_i)}{(W_i + W_j)}$$

where:

t=Retention time of solute; and the subscripts i and j designate two different peaks and where t_j is larger than t_i; and

W=Width of peak at baseline as determined by extrapolating the relative straight sides to the baseline. Both t and W must be measured in the same units.

Resolution between the cyclosporine peak and any other peak must be at least 1.1.

(g) *Procedure.* Using the equipment, columns, mobile phase, operating conditions and the working standard and sample solutions listed in paragraphs (a), (b), (c), (d), and (e) of this section, inject 20 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard solution has been completed, inject 20 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(h) *Calculations.* Calculate the cyclosporine content of cyclosporine and its dosage forms as directed in the individual monographs.

[49 FR 22631, May 31, 1984; 49 FR 27489, July 5, 1984]

§ 436.347 High-pressure liquid chromatographic assay for cefoxitin.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) *One percent potassium phosphate buffer, pH 6.0.* Prepare as described in § 436.101(a)(1).

(2) *Mobile phase.* Mix distilled water:glacial acetic acid:acetonitrile (800:10:190). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.0 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. The minimum between peaks must be no more than 2 millimeters above the baseline.

(d) *Preparation of working standard and sample solutions.* Use the working standard and sample solutions prepared as described in the individual monographs for the drug being tested.

(e) *Procedure.* Using the equipment, reagents, and operating conditions as described in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the cefoxitin content as described in the individual monographs for the drug being tested.

[49 FR 47827, Dec. 7, 1984]

§ 436.348 High-pressure liquid chromatographic assay for ceforanide.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4-centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed wth octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX. A particular column used for analysis of ceforanide should not be used for the analysis of other drugs.—

(b) *Mobile phase.* Mix 18.0 milliliters of 10 percent aqueous tetrabutylammonium hydroxide and 8.56 milliliters of 11*N* potassium hydroxide. Add the mixture to approximately 700 milliliters of distilled water. Add 200 milliliters of reagent grade methanol. Adjust the pH of the mixture to pH 7.0 with concentrated phosphoric acid and dilute to 1,000 milliliters with distilled water. Prepare fresh daily. Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(c) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale.

(d) *Preparation of working standard and sample solutions*—(1) *Preparation of working standard solution.* Prepare a solution containing 1,000 micrograms of ceforanide activity per milliliter in mobile phase. Inject working standard solution within 5 minutes after dissolution.

(2) *Preparation of sample solution.* Prepare the sample solution as directed in the individual monograph for the drug being tested. Inject sample solution within 5 minutes after dissolution.

(e) *Procedure.* Use the equipment, mobile phase, operating conditions, and working standard and sample solutions described in paragraphs (a), (b), (c), and (d) of this section, and proceed as directed in paragraph (e)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile

phase followed by three replicate injections of 10 microliters each of the working standard solution. Allow an elution time sufficient to obtain satisfactory separation of expected components after each injection. Record the peak responses and calculate the tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (e)(2) of this section if the following minimum performance requirements have been met:

(i) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.2;

(ii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,900 theoretical plates;

(iii) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(iv) *Capacity factor.* The capacity factor is satisfactory if it is not less than 1.8 and not more than 5.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (e)(2) of this section.

(2) *Determination of the chromatogram.* Inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the ceforanide content as directed in the individual monograph for the drug being tested.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984]

§ 436.349 High-pressure liquid chromatographic assay for L-lysine in ceforanide for injection.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A suitable pump capable of reproducibly delivering a liquid to a pressure of 5,000 pounds per square inch;

(2) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(3) A suitable recorder;

(4) A suitable integrator; and

(5) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 micrometers to 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents*—(1) 2,4-Dinitrofluorobenzene solution. Weigh accurately approximately 760 milligrams of 2,4-dinitrofluorobenzene into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute ethyl alcohol.

(2) Tris (hydroxymethyl) aminomethane (THAM) solution. Weigh accurately approximately 1.44 grams of THAM into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water.

(c) *Mobile phase.* Mix methanol and water (62:38), and adjust to pH 3.0 with glacial acetic acid.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.5 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the standard that is at least 50 percent of scale with a typical chart speed of 0.2 inch per minute.

(e) *Preparation of standard and sample solutions*—(1) *Preparation of standard solution.* Weigh accurately approximately 36 milligrams of *L*-lysine used as the standard into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the *L*-lysine solution into a 10-milliliter volumetric flask, add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(2) *Preparation of sample solution.* Weigh accurately approximately 150

milligrams of the sample, ceforanide for injection, into a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 2.0 milliliters of the sample solution into a 10-milliliter volumetric flask, add 2.0 milliliters of THAM solution and 3.0 milliliters of 2,4-dinitrofluorobenzene solution. Cap tightly and mix well. Place the flask in a 50° C water bath for 30 minutes. Remove from water bath, allow the flask to cool to room temperature, and dilute to volume with methanol. Mix well.

(f) *Procedure.* Use the equipment, reagents, mobile phase, operating conditions, and standard and sample solutions described in paragraphs (a), (b), (c), (d), and (e) of this section, and proceed as directed in paragraph (f)(1) of this section.

(1) *System suitability test.* Equilibrate and condition the column by passage of about 10 to 15 void volumes of mobile phase followed by three replicate injections of 20 microliters each of the standard solution. Allow an elution time sufficient to obtain satisfactory separation of the expected components after each injection. Record the peak responses and calculate the resolution factor, tailing factor, efficiency of the column, coefficient of variation, and capacity factor as described for system suitability tests in the U.S.P. XX General Chapter 621 chromatography. Proceed as directed in paragraph (f)(2) of this section if the following minimum performance requirements have been met:

(i) *Resolution factor.* The resolution factor between the peak for derivatized *L*-lysine and from the peak for the dinitrofluorobenzene derivatizing reagent is satisfactory if it is not less than 4.5;

(ii) *Tailing factor.* The tailing factor is satisfactory if it is not more than 1.3;

(iii) *Efficiency of the column.* The efficiency of the column is satisfactory if it is greater than 1,500 theoretical plates;

(iv) *Coefficient of variation.* The coefficient of variation of at least three replicate injections is satisfactory if it is not more than 1.5 percent; and

(v) *Capacity factor.* The capacity factor is satisfactory if it is not less than 4 and not more than 6.

If the minimum performance requirements are not met, adjustments must be made to the system to obtain satisfactory operation before proceeding as described in paragraph (f)(2) of this section.

(2) *Determination of the chromatogram.* Inject 20 microliters of the standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of the expected components. After separation of the standard solution is completed, inject 20 microliters of the sample solution into the chromatograph and repeat the procedure described for the standard solution.

(g) *Calculations.* Calculate the percent of *L*-lysine per milligram of ceforanide for injection as follows:

$$\text{Percent of } L\text{-lysine} = \frac{A_u \times P_s}{A_s \times C_u \times 10}$$

where:

A_u = Area of the *L*-lysine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the *L*-lysine peak in the chromatogram of the *L*-lysine standard:

P_s = *L*-lysine content in the *L*-lysine standard solution in micrograms per milliliter; and

C_u = Milligrams of sample per milliliter of sample solution.

[49 FR 25846, June 25, 1984; 49 FR 34347, Aug. 30, 1984; 49 FR 40006, Oct. 12, 1984]

§ 436.350 High-performance liquid chromatographic assay for cefonicid.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in

that monograph, the inlet of this column may be connected to a guard column 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements as follows:

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$ = Width of peak at 5 percent height; and

f = Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute; and

w_h=Peak width at half-height.

(3) *Resolution factor.* Calculate the resolution factor (R), between desacetyl cefonicid and cefonicid, as follows:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where:

t_1=Retention time of desacetyl cefonicid;

t_2=Retention time of cefonicid; and

w_1 and w_2=Widths of the bases of the corresponding peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[49 FR 34347, Aug. 30, 1984, as amended at 49 FR 44460, Nov. 7, 1984; 50 FR 29209, July 18, 1985]

§ 436.351 High-performance liquid chromatographic assay for amoxicillin and clavulanic acid.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 10 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. The retention times for amoxicillin and clavulanic acid are about 2.1 and 1.0 minutes, respectively, under these prescribed conditions. Record the peak responses and calculate the prescribed system suitability requirements as follows:

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factors for the amoxicillin and clavulanic acid peaks.* Calculate the tailing factors (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of amoxicillin or clavulanic acid peaks; and

w_h=Corresponding peak width at half-height.

(3) *Resolution factor.* Calculate the resolution factor (R) as follows:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where:

t_1=Retention time of amoxicillin peak;

t_2=Retention time of clavulanic acid peak; and

w_1 and w_2=Widths of the bases of the corresponding peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

(4) *Coefficient of variation (Relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[49 FR 39671, Oct. 10, 1984]

§ 436.352 High-performance liquid chromatographic assay for determining clavam-2-carboxylate content in clavulanate potassium.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, approximately 30 centimeters in length, packed with a material as defined in the monograph for the drug being tested.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 0.5 milliliter per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the mobile phase and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. The retention times for clavam-2-carboxylic acid and clavulanic acid are about 10 and 14 minutes, respectively, under these prescribed conditions. The sample solution should be injected at least in duplicate and an average should be taken. For each such series of samples injected, two injections of standard should be made, one before and one after the sample series, and an average should be taken. Record the peak responses and calculate the prescribed system suitability requirements as follows:

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of clavam-2-carboxylic acid peak; and

w_h=Corresponding peak width at half-height.

(3) *Resolution factor.* Calculate the resolution factor (R) as follows:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where:

t_1=Retention time of clavam-2-carboxylic acid peak;

t_2=Retention time of clavulanic acid peak; and

w_1 and w_2=Widths of the bases of the corresponding peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

(4) *Coefficient of variation (Relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[49 FR 39671, Oct. 10, 1984]

§ 436.353 High-performance liquid chromatographic assay for amdinocillin.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements as described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column by either of the following formulas:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2; \text{ or}$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute;

w_h=Peak width at half-height; and

W=Width of the base of the peak obtained by extrapolating the relatively straight sides of the peak to the baseline.

(3) *Resolution factor.* Calculate the resolution factor (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:
t_{Rj}=Retention time for a solute eluting after i (t_{Rj} is larger than t_{Ri});
t_{Ri}=Retention time for any solute;
w_i=Width of peak at baseline for any solute; and
w_j=Width of peak at baseline for any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}}\left(\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right)^{1/2}$$

where:
$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[50 FR 7764, Feb. 26, 1985; 50 FR 10220, Mar. 14, 1985; 50 FR 18243, Apr. 30, 1985]

§ 436.354 High-performance liquid chromatographic assay for ceftriaxone.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested.

(b) *Procedure.* Perform the assay at the temperature specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute, Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale. Use the apparatus described in paragraph (a) of this section; and also, use the system suitability requirements, reagents, working standard, test and sample solutions, and calculations as directed in the individual monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of 20 microliters each of the test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements as described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the apparatus and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Capacity factor.* Calculate the capacity factor (k) as follows:

$$k = \frac{t_R - t_M}{t_M}$$

where:
t_R=Retention time of solute; and
t_M=Retention time of solvent or unretained substance.

(2) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:
t_{Rj}=Retention time for a solute eluting after i (t_{Rj} is larger than t_{Ri});
t_{Ri}=Retention time for any solute;
w_i=Width of peak at baseline for any solute; and
w_j=Width of peak at baseline for any solute eluting after i.

(3) *Asymmetry factor.* Calculate the asymmetry factor (A_s

$$A_s = \frac{a+b}{2a}$$

where:
a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from point of maximum peak height to point of descent.

(4) *Efficiency of the column.* Calculate the efficiency of the column (reduced plate height) (h_r

(i)

$$n = 5.545\left(\frac{t_R}{W_h}\right)^2;$$

(ii)

$$h = \frac{L}{n}; \text{ and}$$

(iii)

$$h_r = \frac{h}{d_p}.$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute;

w_h=Peak width at half-height;

h=Efficiency, as height equivalent to one theoretical plate;

L=Length of column; and

d_p=Average diameter of particle in column.

(5) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

The complete operating system is acceptable for assay if it meets the system suitability requirements of the monograph for the drug being tested. If the complete operating system is acceptable, proceed as described in paragraph (b) of this section using the sample solution in lieu of the test solution. Calculate the drug content as described in the individual monograph for the drug being tested.

[50 FR 9999, Mar. 13, 1985]

§ 436.355 High-performance liquid chromatographic assay for ticarcillin-clavulanic acid.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being testing;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 10 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. The clavulanic acid peak is sharp and the chromatograms of standard and sample solutions show baseline separations between it and any neighboring peaks. The retention times for clavulanic acid and ticarcillin are approximately 3 minutes and 14 minutes, respectively. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factors for the ticarcillin and clavulanic acid peaks.* Calculate the tailing factors (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left(\frac{t_R}{W_h}\right)^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of ticarcillin or clavulanic acid peaks; and

w_h=Corresponding peak width at half-height.

(3) *Resolution factor.* Calculate the resolution factor (R) as follows

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where:

t_1=Retention time of clavulanic acid peak;

t_2=Retention time of ticarcillin peak; and

w_1 and w_2=Widths of the bases of the corresponding peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.

When using the method to assay clavulanic acid alone, the resolution factor is not applicable.

(4) *Coefficient of variation (Relative standard deviation).* Calculate the coefficient of variation (S_R

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[50 FR 33517, Aug. 20, 1985; 50 FR 43384, Oct. 25, 1985]

§ 436.356 High-performance liquid chromatographic assay for ceftazidime.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute.

Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution for the system suitability test. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left(\frac{t_R}{w_h}\right)^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute; and

w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj}=Retention time of a solute eluting after i(t_{Rj} is larger than t_{Ri});

t_{Ri}=Retention time of any solute;

w_i=Width of peak at baseline measured by extrapolating the relatively straight sides to the baseline of any solute; and

w_j=Width of peak at baseline measured by extrapolating the relatively straight sides to the baseline of any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[50 FR 48397, Nov. 25, 1985]

§ 436.357 Atomic absorption test for sodium carbonate content.

(a) *Equipment.* A suitable atomic absorbance spectrophotometer equipped with:

(1) A suitable sodium hollow-cathode discharge lamp;

(2) An oxidizing air-acetylene flame;

(3) A nebulizer-burner system;

(4) An optical dispersing device capable of isolating a resonance line of sodium from other wavelengths produced by the emission source; and

(5) A suitable radiation detector.

(b) *Ionization buffer.* Dissolve and dilute 19.07 grams of potassium chloride in distilled water to 1,000 milliliters.

(c) *Preparation of reference standard and sample solutions*—(1) *Reference*

standard solution. Accurately weigh approximately 140 milligrams of sodium chloride, which has been previously dried for 40 to 50 minutes at a temperature of 500 to 650 ° C. Dissolve and dilute with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter. Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter.

(2) *Sample solution.* Dilute the stock sample solution, prepared as directed in the monograph for the drug being tested, with distilled water to obtain a solution containing 5.5 micrograms of sodium per milliliter (estimated). Mix 10 milliliters of this solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter (estimated).

(3) *Procedure.* Determine the atomic absorbance of the reference standard and sample solutions at a wavelength of 589 nanometers, using the atomic absorbance spectrophotometer and a reagent blank prepared by diluting 10 milliliters of ionization buffer to 100 milliliters with distilled water.

(d) *Calculations.* Calculate the percent sodium carbonate (S) as follows:

$$\text{Percent sodium carbonate} = \frac{A_u \times P_s \times 2.304}{A_s \times C_u \times 10}$$

where:

A_u=Absorbance of sodium in the sample solution;

A_s=Absorbance of sodium in the reference standard solution;

P_s=Sodium concentration in the reference standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

[50 FR 48398, Nov. 25, 1985, as amended at 54 FR 20785, May 15, 1989]

§ 436.358 High-performance liquid chromatographic assay for pyridine.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 15 to 25 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the pyridine content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 25 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed for the system suitability test by five replicate injections of the same volume (between 10 and 20 microliters) of the system suitability test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor for the pyridine peak (*T*), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Resolution*. Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj}=Retention time of *t*-butyl ceftazidime;

t_{Ri}=Retention time of pyridine;

w_i=Width of pyridine peak at the baseline measured by extrapolating the relatively straight sides to the baseline; and

w_j=Width of *t*-butyl ceftazidime peak at the baseline measured by extrapolating the relatively straight sides to the baseline.

(3) *Coefficient of variation (relative standard deviation)*. Calculate the coefficient of variation for the pyridine peak (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[50 FR 48398, Nov. 25, 1985; 50 FR 53308, Dec. 31, 1985]

§ 436.360 Gel permeation chromatographic assay for high molecular weight polymer.

(a) *Equipment*. A suitable gel permeation chromatograph equipped with.

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 50 centimeters long and 9 millimeters internal diameter, packed with a material as defined in the monograph for the drug being tested.

(b) *Procedure*. Perform the assay and calculate the high molecular weight polymer content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 10 percent of scale with a typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of mobile phase for not less than 18 hours, removing any voids that may form at the top of the column, followed by five replicate injections of the same volume (100 microliters) of the blue dextran system suitability test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test*. Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor*. Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and

f=Horizontal distance from point of ascent to

a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left(\frac{t_R}{w_h}\right)^2$$

where:
n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:
$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using two injections of the same volume (100 microliters) of the working standard solution followed by one injection of the same volume (100 microliters) of the sample solution.

[50 FR 48398, Nov. 25, 1985; 51 FR 1367, Jan. 13, 1986]

§ 436.361 High-performance liquid chromatographic assay for aztreonam.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:
$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;

t_R=Retention time of solute; and

w_h=Peak width at half-height.

(3) *Resolution*. Calculate the resolution (R) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj}=Retention time of a solute eluting after i (t_{Rj} is larger than t_{Ri});

t_{Ri}=Retention time of any solute;

w_i=Width of peak at baseline of any solute; and

w_j=Width of peak at baseline of any solute eluting after i.

(4) *Coefficient of variation (relative standard deviation)*. Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of N individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[52 FR 4611, Feb. 13, 1987; 52 FR 8550, Mar. 18, 1987]

§ 436.362 Thin-layer chromatographic test for free erythromycin content in erythromycin estolate bulk.

(a) *Equipment*—(1) *Chromatography tank*. A rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top.

(2) *Plates*. Use a 20- by 20-centimeter precoated silica gel 60 F–254 thin-layer chromatography plate. Before using, place the plate in an unlined developing chamber containing approximately 100 milliliters of anhydrous methanol and allow the solvent front to travel to the top of the plate, marking the direction of travel. Remove the plate and allow to drip dry. Store in a dry place.

(b) *Reagents*—(1) *Developing solvent*. Mix 15 milliliters of chloroform and 85 milliliters of anhydrous methanol. Use fresh developing solvent for each test.

(2) *Spray solution*. Dissolve 150 milligrams of xanthydrol in a mixture of 7.5 milliliters of glacial acetic acid and 92.5 milliliters of 37 percent hydrochloric acid.

(c) *Preparation of spotting solutions*—(1) *Sample solution*. Prepare a solution of the sample in anhydrous methanol to contain 10 milligrams per milliliter.

NOTE: It is advisable to prepare the sample and standard solutions immediately before spotting to minimize the possibility of degradation in solution.)

(2) *Standard solution*. Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 1 milligram per milliliter. Weigh 99.5, 99.0, and 97.0 milligrams of erythromycin estolate (propionyl erythromycin lauryl sulfate) reference standard and transfer to separate 10-milliliter volumetric flasks. To these flasks add 0.5, 1.0, and 3.0 milliliters, respectively, of the 1-milligram-per-milliliter solution of erythromycin base reference standard and dilute to volume with anhydrous methanol. These solutions contain, respectively, 0.5 percent, 1.0 percent, and 3.0 percent erythromycin base in erythromycin estolate. Prepare a solution of erythromycin estolate reference standard in anhydrous methanol to contain 10 milligrams per milliliter. Prepare a solution of erythromycin base reference standard in anhydrous methanol to contain 0.1 milligram per milliliter.

(d) *Procedure*. Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a

plate as follows: On a line 2.0 centimeters from the base of the thin-layer plate, apply 1.0 microliter of each of the following solutions:

(1) 10-milligrams-per-milliliter solution of erythromycin estolate reference standard, equivalent to 10 micrograms of erythromycin estolate;

(2) 0.5 percent base-in-estolate solution, equivalent to 0.05 microgram of base and 9.95 micrograms of estolate;

(3) 1.0 percent base-in-estolate solution, equivalent to 0.10 microgram of base and 9.90 micrograms of estolate;

(4) 3.0 percent base-in-estolate solution, equivalent to 0.30 microgram of base and 9.70 micrograms of estolate;

(5) 0.1-milligram-per-milliliter solution of erythromycin base reference standard, equivalent to 0.1 microgram of erythromycin base; and

(6) Sample solution, equivalent to 10 micrograms of erythromycin estolate. Allow the spots to dry. Place the plate directly in the chromatograph tank. Cover and seal the tank. Allow the solvent front to travel a distance of 7 centimeters (about 27 minutes). Remove the plate from the tank, and allow it to air dry under a hood. With the plate still under the hood, spray uniformly with the spray solution. Heat the sprayed plate in an oven at 100 °C for 5 minutes. (CAUTION: Avoid exposure to the acid fumes while removing the plate from the oven.)

(e) *Evaluation.* Erythromycin base and erythromycin estolate appear as reddish-violet spots on the sprayed and heated plate. Better visualization of the erythromycin base spots may be gained by viewing the plate under long-wavelength (366 nanometers) ultraviolet light, erythromycin base appearing as dark spots on a yellow-green fluorescent background. Erythromycin base has an R_f value of about 0.3. Erythromycin estolate has an R_f value of about 0.7. Compare the size and intensity of any erythromycin base spots in the sample lane with the erythromycin base spots in the erythromycin base reference standard lane and in the 0.5 percent, 1.0 percent, and 3.0 percent base-in-estolate lanes, and report the percentage of erythromycin base (free erythromycin) in the sample. For a more accurate determination of free erythromycin content, it may be necessary to repeat the test using a different set of standards.

[53 FR 1919, Jan. 25, 1988]

§ 436.363 High-performance liquid chromatographic assay for cefmenoxime.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 18-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 30 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested with a flow rate not to exceed 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the apparatus described in paragraph (a) of this section; and the reagents and working standard and sample solutions described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by 5 replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Using the apparatus and procedure described in

this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (T), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:
$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (n) of the column as follows:

$$n = 5.545\left[\frac{t_R}{W_h}\right]^2$$

where:
n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (R) as follows:

$$R = \frac{2\left(t_{\mathrm{RJ}} - t_{\mathrm{R}i}\right)}{w_{\mathrm{i}} + w_{\mathrm{J}}}$$

where:
t_{RJ}=Retention time of a solute eluting after i (t_{RJ} is larger than t_{Ri});
t_{Ri}=Retention time of any solute;
w_{i}=Width of peak at baseline of any solute; and
w_{J}=Width of peak at baseline of any solute eluting after i.

(4) *Coefficient of variation (Relative standard deviation).*

Calculate the coefficient of variation (S_{R}) in percent) as follows:

$$\underline{S_R} = \frac{100}{\underline{\overline{X}}}\left[\frac{\sum_{i=1}^{N}(\underline{X_i} - \underline{\overline{X}})^2}{\underline{N} - 1}\right]^{\frac{1}{2}}$$

where:
X is the mean of N individual measurements of X_{i}. If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

[53 FR 13401, Apr. 25, 1988; 53 FR 19368, May 27, 1988]

§ 436.364 Atomic absorption test for sodium carbonate content of cefmenoxime hydrochloride for injection.

(a) *Apparatus.* A suitable atomic absorbance spectrophotometer equipped with:

(1) A suitable sodium hollow-cathode discharge lamp;

(2) An oxidizing air-acetylene flame;

(3) A nebulizer-burner system;

(4) An optical dispersing device capable of isolating a resonance line of sodium from other wavelengths produced by the emission source; and

(5) A suitable radiation detector.

(b) *Reagents.* Ionization buffer: Dissolve 19.07 grams of potassium chloride in distilled water and dilute to 1,000 milliliters.

(c) *Preparation of reference standard and sample solutions*—(1) *Reference standard solution.* Accurately weigh approximately 140 milligrams of sodium chloride which has been previously dired for 40 to 50 minutes at a temperature of 500 to 650 ° C. Dissolve and dilute with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter. Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter.

(2) *Sample solution.* Dilute the sample solution used in § 442.222(b)(1)(ii)(B)(*1*) of this chapter, with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter (estimated). Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter (estimated).

(3) *Procedure.* Determine the atomic absorbance of the reference standard and sample solutions at a wavelength

of 589 nanometers, using the atomic absorbance spectrophotometer and a reagent blank prepared by diluting 10 milliliters of ionization buffer to 100 milliliters with distilled water.

(d) *Calculations.* Calculate the percent sodium carbonate as follows:

$$\text{Percent sodium carbonate} = \frac{A_u \times P_s \times 100 \times 0.9068 \times d}{A_s \times C_u}$$

where:

A_u=Absorbance of sodium in the sample solution;

A_s=Absorbance of sodium in the reference standard solution;

P_s=Milligrams of sodium chloride per milliliter of the reference standard solution;

C_u=Milligrams of sample per milliliter of sample solution; and

d= Dilution factor of the sample.

[53 FR 13401, Apr. 25, 1988]

§ 436.365 Thin layer chromatographic identity test for rifampin.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover, lined with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20×20 centimeter thin layer chromatography plates coated with silica gel 60 F–254 or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform and methanol in volumetric proportions of 90:10, respectively.

(c) *Spotting solutions*—(1) *Preparation of working standard solution.* Dissolve approximately 50 milligrams of rifampin working standard in 5 milliliters of chloroform.

(2) *Preparation of sample solution.* Dissolve the contents of a sample vial in 60 milliliters of chloroform.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate. Prepare a plate as follows: On a line 2.5 centimeters from the base of the thin layer chromatography plate and at intervals of 2.0 centimeters, spot 3 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply 3 microliters of the sample solution to points 2 and 3. After all the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank. Allow the solvent front to travel about 7 centimeters from the starting line. Remove the plate from the tank and air dry.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the red spots are from the starting line. Divide the latter by the former to calculate the R_f value.

[54 FR 38375, Sept. 18, 1989; 54 FR 42886, Oct. 18, 1989]

§ 436.366 High-performance liquid chromatography assay for determining chromatographic purity of vancomycin.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers or preferably 280 nanometers;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) A 25-centimeter analytical column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles; 5 micrometers in diameter.

(b) *Reagents*—(1) *0.2 percent triethylammonium phosphate buffer.* To 2,000 milliliters of distilled water, either add 4 milliliters of triethylamine or 4 grams of triethylammonium chloride. Adjust the pH to 3.2 with phosphoric acid.

(2) *Sample solvents.* (i) Vancomycin hydrochloride: Mobile Phase A.

(ii) Vancomycin base: 5 milliliters Mobile Phase A; add 0.1*N* HCl dropwise with swirling until sample dissolves: dilute to volume with Mobile Phase A.

(c) *Mobile Phases*—(1) *Mobile Phase A.* Add 70 milliliters of acetonitrile and 10 milliliters of tetrahydrofuran to 920 milliliters of 0.2 percent triethylammonium phosphate buffer and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase, briefly, just prior to its introduction into the chromatographic pumping system.

(2) *Mobile Phase B.* Add 290 milliliters of acetonitrile and 10 milliliters of tetrahydofuran to 700 milliliters of 0.2 percent triethylammonium phosphate buffer and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase, briefly, just prior to its introduction into the chromatographic pumping system.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of about 2.0 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the main peak (Vancomycin B) that is at least 50 percent of scale. The run time is 30 minutes per injection and the gradient conditions are as follows: (0, 12, 12.5, 8, 0, 2)

Time (minutes)	Mobile phase A (percent)	Mobile phase B (percent)	Gradient condition
0	100	0	Initial conditions.
12	100	0	Isocratic region.
20	0	100	Linear ramp.
22	0	100	Isocratic region.
23	100	0	Return to initial.
30	100	0	Reequilibration.

(e) *Preparation of resolution and sample solutions*—(1) *Resolution solution.* Prepare a solution of vancomycin hydrochloride reference standard in water containing 0.5 milligram per milliliter. Heat at 65 °C for 24 hours and allow to cool. This procedure generates two desamido-vancomycin isomers. The first desamido isomer elutes during the isocratic period and before the vancomycin B peak; the second desamido isomer elutes during the gradient ramp and is used to demonstrate the effective performance of this stage.

(2) *Sample preparation.* In a volumetric flask either dissolve a representative sample or dilute a representative portion with sample solvent to give a sample preparation containing approximately 10 milligrams per milliliter. Pipet 2 milliliters of this sample solution into a separate 50-milliliter volumetric flask and dilute to volume with sample solvent to give a diluted sample preparation containing approximately 0.4 milligram per milliliter.

(f) *Procedure.* Optimize chromatographic conditions under isocratic conditions by equilibrating the system while pumping 100 percent mobile phase A through the column. Inject 20 microliters of the resolution solution onto the column and record the chromatogram. Adjust the acetonitrile concentration of mobile phase A as needed to provide a retention time for vancomycin B of 7.5 to 10.5 minutes. Use the resolution solution to perform the system suitability tests. The elution order is resolution compound 1, vancomycin B, resolution compound 2. Return the system to the initial gradient operating conditions. Separately inject 20 milliliters of each diluted (0.4 milligram per milliliter) and concentrated (10 milligrams per milliliter) sample solution onto the column and record each chromatogram.

(g) *System suitability test.* Using the resolution solution described in paragraph (e)(1) of this section, test the performance of the chromatographic system as follows:

(1) *Asymmetry factor.* Calculate the asymmetry factor (*A*), measured at a point that is 10 percent of the vancomycin B peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor (A_r) is satisfactory if it is not less than 0.8 and not more than 1.8.

(2) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) calculate the reduced plate height (h_r) for the vancomycin B peak as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;

n=Number of theoretical plates; and

d_p=Average diameter of the particles in the column in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 40 for the vancomycin B peak in the resolution solution.

(3) *Resolution.* The resolution (R) between the vancomycin B peak and the peak for resolution compound 1 is not less than 3.0. Resolution compound 2 is eluted between 3 and 6 minutes after the start of the period when the percentage of mobile phase B is increasing from 0 percent to 100 percent.

(4) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of five replicate injections of the resolution solution is calculated as described in § 436.216(c)(4) is satisfactory if it is not more than 2.0 percent.

(5) *Capacity factor (k).* Calculate the capacity factor (k) for vancomycin B as follows:

$$k = \frac{t_r - t_m}{t_m}$$

where:

t_r=Retention time of solute; and

t_m=Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;

L=Column length in centimeters;

0.75=Average total column porosity; and

F=Flow rate in milliliters per minute.

The capacity factor (k) for vancomycin B is satisfactory if it is not less than 2.6 and not more than 3.3.

When the system suitability requirements have been met, then proceed as described in paragraph (f) of this section. Alternate chromatographic conditions are acceptable provided that the system suitability parameters are met. However, the sample preparation described in paragraph (e)(2) of this section should not be changed.

(h) *Calculations.* (1) Calculate the percentage of vancomycin B in the specimen as follows:

$$\text{Percentage of vancomycin B} = \frac{A_B}{A_{Total}} \times 100 \text{ percent}$$

where:

A_B=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) sample solution; and

A_{Total}=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

(2) Calculate the percentage of each other peak as follows:

$$\text{Percentage of related substance } (i) = \frac{[A_{i/25}]}{A_{Total}} \times 100 \text{ percent}$$

where:

A_i=Area of any given peak, other than the main peak in the concentrated solution (10 milligrams per milliliter); and

A_{Total}=Area of the vancomycin B peak in the dilute (0.4 milligram per milliliter) solution+[Area of the total related substances peaks (exclude the area of the vancomycin B peak) in the concentrated solution (10 milligrams per milliliter) divided by 25].

[54 FR 20383, May 11, 1989; 54 FR 25849, June 20, 1989]

§ 436.367 Thin-layer chromatographic identity test for cephalexin hydrochloride.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23 × 23 × 9 centimeters, with a glass solvent trough in the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3 MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatographic plates coated with silica gel 60F–254 or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix ethylacetate, acetonitrile, water and glacial acetic acid in volumetric proportions of 42:14:18:14, respectively.

(c) *Preparation of the spotting solutions.* Prepare a solution of the sample containing 25 milligrams per milliliter of cephalexin hydrochloride in water. Prepare a solution of cephalexin monohydrate reference material at a concentration of 25 milligrams per milliliter. Add water and 0.1*N* hydrochloric acid in a dropwise mode until the material is completely dissolved.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot approximately 5 microliters of the standard solution to points 1 and 3 and approximately 5 microliters of the sample solution to point 2. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank. Allow the solvent front to travel approximately 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry.

(e) *Evaluation.* View the dry plate under ultraviolet light (254 nanometers). Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values of approximately 0.35.

[54 FR 48860, Nov. 28, 1989; 54 FR 51816, Dec. 18, 1989]

§ 436.368 Thin layer chromatographic identity test for cefprozil.

(a) *Equipment*—(1) *Chromatography tank.* Use a glass rectangular tank approximately 23 x 23 x 9 centimeters lined with filter paper and equipped with a tight-fitting cover.

(2) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with silica gel GF to a thickness of 250 microns.

(b) *Reagents*—(1) *Diluent.* Mix 0.1N HCl and acetone in volumetric proportions of 1:4.

(2) *Developing solvent.* Mix n-butanol, glacial acetic acid and water in volumetric proportions of 60:20:20.

(3) *Detection reagent.* Iodine vapor.

(c) *Assay solutions*—(1) *Reference standard solution.* Dissolve 50 milligrams of cefprozil (Z) reference standard in 10 milliliters of diluent.

(2) *Sample solution.* Place an amount of sample containing approximately 50 milligrams of cefprozil in a 20-milliliter glass stoppered vial. Add 10 milliliters of diluent. Shake for 5 minutes and allow the solids to settle.

(d) *Procedure.* Pour a suitable quantity of the developing solvent into a glass, chromatographic tank lined with filter paper and allow to equilibrate for 1 hour. On a line 2 centimeters from the bottom edge of the plate, spot 10 microliters each of the reference solution and sample solution. Draw a line indicating the distance to which the developing solvent must travel at a point 12 centimeters from the bottom edge of the plate. Place the plate in the tank and allow the solvent to migrate to the finishing line. Remove the plate and air dry in a fume hood. Place the dried plate in a chamber containing iodine vapors.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the spots are from the starting line. Divide the latter by the former to calculate the R_f value. The identity test is positive if the sample solution produces a yellow spot at the same R_f value and has the same appearance as the spot obtained for the reference solution. The R_f value for cefprozil (Z) is approximately 0.45. Cefprozil (E), has an R_f value of approximately 0.47.Cefprozil (Z) is ''absent'' if the above test is performed and no spots, which correspond to those from the reference solution, are obtained for the sample.

[58 FR 26660, May 4, 1993]

§ 436.369 Thin layer chromatography test for free *N*-isobutylpiperidone content in rifabutin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover.

(2) *Iodine vapor chamber.* A rectangular tank, approximately 23 X 23 X 9 centimeters, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 X 20 centimeter thin layer chromatography plates coated with silica gel 60F 254 or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Developing solvent.* Mix petroleum ether (b.p. 60 to 80 ° C) and acetone in volumetric proportions of 100:30, respectively.

(2) *Spray solution.* Prepare a 1 percent solution of soluble starch in water (containing 0.01 percent mercuric iodide).

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Prepare a solution of the rifabutin sample in 1:1 chloroform/methanol to contain 10 milligrams per milliliter.

(2) *Standard solution.* Prepare a solution of *N*-isobutylpiperidone standard in 1:1 chloroform/methanol to contain 1 milligram per milliliter. Transfer aliquots of 0.5, 1.0, 2.0, 5.0, and 10.0 milliliters into separate 100-milliliter volumetric flasks and dilute to volume with 1:1 chloroform/methanol. These solutions contain, respectively, the equivalent of 0.05, 0.1, 0.2, 0.5, and 1.0 percent of *N*-isobutylpiperidone.

(d) *Procedure.* Pour 100 milliliters of developing solvent into the glass trough on the bottom of the unlined chromatography tank. Cover and seal the tank. Allow it to equilibrate while the plate is being prepared. Prepare a plate as follows: on a line 2.0 centimeters from the base of the thin layer chromatography plate, and at intervals of 2.0 centimeters, apply 10 microliters of each of the standard solutions and the sample solution prepared as directed above. After the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank, allow the solvent front to travel about 15 centimeters from the starting line and then remove the plate from the tank. Air dry the plate. Warm the iodine vapor chamber to vaporize the iodine crystals and place the dry plate in the iodine vapor chamber until the spots are visible (usually about 5 minutes). Remove the plate from the iodine vapor chamber and spray with 1 percent starch solution.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. *N*-isobutylpiperidone has an R_f value of about 0.3. Rifabutin has an R_f value of about 0.1. Compare the size and intensity of any *N*-isobutylpiperidone spots in the sample lane with the *N*-isobutylpiperidone spots in the standard lanes, and report the percentage of *N*-isobutylpiperidone in the sample.

[59 FR 40806, Aug. 10, 1994]

§ 436.370 Spectrophotometric identity test for rifabutin capsules.

(a) *Equipment.* A suitable spectrophotometer capable of recording the ultraviolet spectrum in the 200 to 400 nanometer range, using suitable quartz cells of 1 centimeter pathlength.

(b) *Preparation of working standard and sample solution*—(1) *Working standard solution.* Suspend approximately 200 milligrams of rifabutin working standard in 20 milliliters of methanol and sonicate for approximately 5 minutes. Filter the resulting solution through a suitable 0.5 micrometer filter. Transfer a 2-milliliter aliquot of the filtered solution to a 100-milliliter volumetric flask and fill to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter.

(2) *Sample solution.* Empty and combine the contents of five capsules. Suspend a quantity of the capsule contents equivalent to 200 milligrams of rifabutin in 20 milliliters of methanol. Sonicate for about 5 minutes and then filter through an appropriate 0.5 micrometer filter. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with methanol. Further dilute with methanol to obtain a solution containing 20 micrograms of rifabutin activity per milliliter (estimated).

(c) *Procedure.* Using a suitable spectrophotometer equipped with 1.0 centimeter cells and methanol as the blank, determine the absorbance spectra of the working standard and sample solutions over the ultraviolet range of 250 to 300 nanometers.

(d) *Evaluation.* Compare the spectrum of the sample to that of the working standard. The identity of the rifabutin capsules is confirmed by quantitative comparison of the two spectra with an

absorbance maximum being observed at about 275 nanometers.

[59 FR 40807, Aug. 10, 1994]

Subpart G—Chemical Tests for Nonantibiotic Active Ingredients

§ 436.400 Thin layer chromatographic identity test for iodochlorhydroxyquin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 9 × 9 × 3.5 inches with a glass solvent trough on the bottom.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix benzene and methanol in volumetric proportions of 90:10.

(c) *Preparation of spotting solutions*—(1) *Sample solution.* Use the sample solution prepared as described in the section for the particular product to be tested.

(2) *Reference solution.* Prepare a solution containing 0.5 milligram of iodochlorhydroxyquin U.S.P. reference standard per milliliter in acetone.

(d) *Procedure.* Pour developing solvent into the glass trough on the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Spot a plate as follows: Apply approximately 10 microliters each of the sample solution and of the reference solution on a line 2.0 centimeters from the base of the silica gel plate and at intervals of not less than 2.0 centimeters. After all spots are thoroughly dry, place the silica gel plate directly into the glass trough of the chromatography tank. Cover and reseal the tank. Allow the solvent front to travel about 15 centimeters from the starting line, remove the plate from the tank, and allow to air dry. Examine under a strong source of ultraviolet light. The sample and standard are visible as dark spots.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values (0.55 to 0.60).

Subpart H—Tests for Specific Antibiotic Dosage Forms

§ 436.500 Penicillin in oil and wax.

(a) *Potency.* Proceed as directed in § 440.80a(b)(1) of this chapter except paragraph (b)(1)(ix) thereof and, in lieu of the directions in § 440.80a(b)(1)(iv) of this chapter prepare sample as follows: Liquefy the sample by warming, thoroughly mix, and withdraw 1.0 milliliter using a sterile syringe equipped with an 18-gauge needle. Transfer to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the separatory funnel vigorously to bring about complete mixing of the material with the ether. Shake with a 25-milliliter portion of 1 percent phosphate buffer at pH 6.0. Remove the buffer layer and repeat the extraction with three 25-milliliter quantites of buffer. Combine the extracts and make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. The sample may also be prepared by transferring aseptically 1.0 milliliter of the penicillin in oil and wax to a blending jar containing 100 milliliters of 1 percent phosphate buffer at pH 6.0. Using a high-speed blender, blend this mixture for 1 minute and then make the proper estimated dilutions in 1 percent phosphate buffer at pH 6.0. If the label represents the potency of the penicillin in oil and wax as 200,000 units per milliliter or less, it is satisfactory if it is 85 percent or more of the potency so represented; if represented as more than 200,000 units per milliliter, it is satisfactory if it is 90 percent or more of the potency so represented.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except using medium B in lieu of medium A.

(c) *Moisture*—(1) *Reagents*—(i) *KarlFischer reagent.* Preserve the reagent in glass-stoppered bottles and use from an all glass automatic burette, protecting the solution from the moisture in the air.

(ii) *Water-methanol solution.* Use methanol containing approximately 1 mg. of water per milliliter. Store the solution in a glass bottle attached to an automatic burette and protect from moisture in the air at all times.

(2) *Standardization of Karl Fischer reagent.* Add a known volume of the Karl Fischer reagent to a suitable titrating vessel which has been previously dried at 105° C. and cooled in a desiccator. Introduce a mechanical stirrer and two platinum electrodes which are connected to a suitable electrometric apparatus for measurement of the endpoint. Start the stirrer and titrate with the water-methanol solution until the endpoint is reached. Calculate the milliliters of Karl Fischer reagent equivalent to each milliliter of water-methanol. Add an accurately weighed quantity of water (approximately 50 milligrams) to a dry titrating vessel, add an excess of the Karl Fischer reagent and back titrate with the water-methanol solution as above. Calculate the milligrams of water equivalent to each milliliter of the Karl Fischer reagent. Standardize the Karl Fischer reagent in this manner daily.

$$e = \frac{W}{V_1 - V_2 f}$$

where:

e=milligrams of water equivalent to 1 ml. Karl Fischer reagent.

w=weight of water in milligrams.

v_1=volume of Karl Fischer reagent used.

v_2=volume of methanol used.

f=volume ratio of Karl Fischer reagent to water-methanol solution.

(3) *Procedure.* Transfer 1.0 milliliter of the penicillin in oil and wax to a dry titrating vessel, add 10 milliliters of dry chloroform and an excess of the Karl Fischer reagent and back titrate with the water-methanol solution until the endpoint is reached. Transfer 10 milliliters of the dry chloroform used to a dry titrating vessel, add an excess of Karl Fischer reagent, and titrate with the water-methanol as above. Calculate the milliliters of Karl Fischer reagent equivalent to 10 milliliters of chloroform.

$$\text{Percent moisture} = \frac{(V_1 - V_2 f - b) \times e \times 100}{s \times 100}$$

where:

b=milliliters Karl Fischer reagent equivalent to 10 ml. of chloroform.

s=volume of the sample in milliliters.

(d) *Measurement of penicillin particle size.* Vigorously shake the container to obtain an even suspension of the penicillin particles and immediately withdraw therefrom approximately 0.5 milliliter of the drug into a clean, dry, tuberculin syringe using a dry 18-gauge needle. Discard approximately the first 5 drops of the mixture extruded from the needle and then extrude approximately 1 minim of the remaining mixture into a test tube containing 3 to 4 milliliters of light mineral oil. Thoroughly mix the contents of the tube and by means of a bacteriological loop (2 millimeters inside diameter, 22 gauge wire), immediately place one loopful of the suspension on each ruled chamber of a bright line hemocytometer. (It is not necessary to use a cover slip.) Confirm by means of the low power objective of the microscope the even distribution of particles over the ruled areas of both chambers and repeat with another loopful of the suspension if even dispersion is not obtained. Use a magnification of 430 or 440 diameters and a calibrated ocular micrometer to measure the penicillin particles. For the purpose of measurement and calculation, the predominant type of crystals observed shall be considered to represent the type of crystals present and the thickness and density of all particles shall be considered constant. Center a large penicillin particle in the microscopic field; measure the particle and all other particles in the field and repeat this operation on other fields until at least 200 particles are measured. Particles of less than 5 microns in length are disregarded. The grouping of the particles by length, the midpoint, the ratio of the midpoints, and the square of the ratio of the midpoints for each group are tabulated below:

Group	Length in microns	Midpoint	Ratio of midpoints	(Ratio)^2
1	5–14	9.5	1.00	1.00
2	15–29	22.0	2.31	5.34
3	30–49	39.5	4.16	17.31
4	50–69	59.5	6.26	39.19
5	70–99	84.5	8.89	79.03
6	100–149	124.5	13.10	171.61
7	150–199	174.5	18.36	337.09
8	200–249	224.5	23.63	558.38
9	250–300	275.0	28.95	838.10

Calculate the percent particles in each group from the total number measured. Determine the percent relative weight for each group as follows:

Plate type particles. Relative weight= (ratio)^2×% of total particles in group.

$$\%\,\text{relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

Rod-shaped particles. In the case of rod-shaped particles measure the width as well as the length.

Relative weight=ratio × average width × % of total particles in group

$$\%\,\text{relative weight} = \frac{\text{Relative weight} \times 100}{\text{Total relative weight}}$$

When examined by the method described in this section not less than 50 percent of the total relative weight of the penicillin in the drug consists of penicillin having a particle size of not less than 50 microns in length.

§ 436.503 Procaine penicillin and buffered crystalline penicillin for aqueous injection.

(a) *Total potency* (*except in single-dose container*), *sterility, moisture, pyrogens, toxicity, pH.* Proceed as directed in § 440.274b(b) of this chapter.

(b) *Buffered crystalline penicillin content*—(1) *Preparation of the solution for assay.* Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution. Dilute a 5.0-milliliter aliquot of this clear solution with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(2) *Iodometric assay for total penicillin in the solution for assay.* Determine the quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) of this chapter.

(3) *Colorimetric determination of procaine penicillin in the solution for assay.* Transfer an aliquot of the solution for assay to a 50-milliliter volumetric flask. Determine the quantity of procaine penicillin in this solution by the following method:

(i) *Reagents*—(*a*) *Sodium nitrite solution.* Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare fresh solution every week and store under refrigeration.

(*b*) *Ammonium sulfamate solution.* Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(*c*) *N-(1-naphthyl)-ethylenediamine solution.* Dissolve 0.1 gram of *N*-(1-naphthyl) ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(*d*) *Standard procaine solution.* Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of procaine penicillin).

(ii) *Standards.* Transfer, respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard solution and 5.0 milliliters of distilled water to each of six 50-milliliter volumetric flasks. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a volume to 5.0 milliliters.

(iii) *Procedure.* To each flask for the standards and the solution for assay add 0.5 milliliter of 4 *N* HCl, 1.0 milliliter of the sodium nitrite solution, 1.0 milliliter of the ammonium sulfamate, and 1.0 milliliter of the *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Make each flask to volume of 50 milliliters with distilled water. Determine the absorbency of the colored solutions at 550 Mμ in a suitable photo electric colorimeter. The instrument is balanced so that the zero concentration reads zero absorbency. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbency.

(4) The content of buffered crystalline pencillin in one dose of the product is calculated as follows:

$$A=(B-C)F,$$

where:

A=buffered crystalline penicillin content of the product.

B=total number of units of penicillin per milliliter as determined in paragraph (b)(2) of this section.

C=number of units of procaine penicillin per milliliter as determined in paragaph (b)(3) of this section.

F=appropriate dilution factor depending on the dilution made in the preparation of the solution for assay.

The content of buffered crystalline penicillin in the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(c) *Procaine penicillin.* The procaine penicillin content of the batch is the difference between the total potency determined by the method described in paragraph (a) or (d) of this section and the content of the buffered crystalline penicillin determined by the method described in paragraph (b) of this section. The procaine penicillin content of the batch is satisfactory when determined by the method described in this paragraph if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a one-dose container.* Wash out the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b)(1) of this section with 1-percent phosphate buffer, pH 6.0. Dilute to give a concentration of approximately 2,000 units per milliliter, and assay by the iodometric method described in § 440.80a (b)(5)(iv)(*a*) of this chapter. Obtain the total potency by adding the number of units found in this solution (units per milliliter × volume) to the number of units found (units per milliliter × volume) in the solution assayed in accordance with paragraph (b)(2) of this section.

§ 436.504 Penicillin-bacitracin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter, except the last sentence of that paragraph. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of bacitracin is satisfactory if it contains not less than 85 percent of the number of units it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.505 Penicillin-streptomycin-bacitracin ointment; penicillin-dihydrostreptomycin-bacitracin ointment; penicillin-streptomycin-bacitracin methylene disalicylate ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate ointment.

(a) *Potency*—(1) *Content of penicillin, streptomycin, and dihydrostreptomycin.* Proceed as directed in § 536.501(a) of this chapter.

(2) *Bacitracin content.* Proceed as directed in § 448.510a(b)(1) of this chapter, except that:

(i) Sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present.

(ii) Use as the test organism the streptomycin dihydrostreptomycin resistant strain of either Micrococcus flavus (ATCC 10240A)[1] or Sarcina subflava (ATCC 7468/d),[1] grown and maintained in media containing 500 micrograms of streptomycin or dihydrostreptomycin per milliliter of media, or calculate from the quantity of streptomycin or dihydrostreptomycin found, using the method prescribed by paragraph (a)(1) of this section, the quantity that would be present when the sample is diluted to contain one unit of bacitracin (labeled potency) per milliliter. Prepare the bacitracin standard curve by adding the calculated quantity of streptomycin or dihydrostreptomycin to each concentration of bacitracin used for

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

the curve. Use this standard curve to calculate the bacitracin content of the sample.

(3) *Bacitracin methylene disalicylate content.* Proceed as directed in paragraph (a)(2) of this section, except prepare the sample as follows: Place a representative portion of the sample (usually approximately 1 gram, accurately weighed) or the entire contents of a single-dose container in blending jar, add 99 milliliters of a 2.0-percent aqueous solution of sodium bicarbonate and 1 milliliter of a 10-percent aqueous solution of polysorbate 80 and blend for 3 minutes in a high-speed blender. Allow the foam to subside, remove an aliquot of the solution, and dilute to 1 unit per milliliter with 1.0-percent phosphate buffer, pH 6.0.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.506 Benzathine penicillin G and buffered crystalline penicillin for aqueous injection.

(a) *Total potency* (*except in single-dose containers*). Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting 1.0 milliliter of the drug suspension with sufficient dimethyl formamide, formamide, or methyl alcohol to dissolve the benzathine penicillin. Make to 100 milliliters with buffer. Shake well and dilute to 1.0 unit per milliliter. If the iodometric method is used, proceed as directed in § 440.55a(b) of this chapter, except in preparing the blank solution dilute 1.0 milliliter of the drug suspension to 250 milliliters with 1-percent phosphate buffer at pH 6.0. In preparing the solution for inactivation dissolve 1.0 milliliter of the drug suspension in approximately 20 milliliters of 0.5 *N* NaOH. Allow to stand for 15 minutes. Dilute to 250 milliliters with distilled water. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask and add 2.0 milliliters 1.2 *N* HCl and 10 milliliters 0.01 *N* iodine.

(b) *Buffered crystalline penicillin content.* Place 1.0 milliliter of the drug suspension in a 10-milliliter volumetric flask and add 20 percent sodium sulfate to make 10 milliliters. Shake well and centrifuge to obtain a clear, or reasonably clear, solution. Dilute a 5.0-milliliter aliquot to 50 milliliters with buffer and proceed as directed in § 440.80a(b)(1) of this chapter to determine the number of units per milliliter of this solution, and from this value calculate the number of units per milliliter of the drug. The content of buffered crystalline penicillin is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(c) *Benzathine penicillin G content.* The benzathine penicillin G content of the batch is the difference between the total potency as described in paragraph (a) or (d) of this section and the content of buffered crystalline penicillin determined by the method prescribed in paragraph (b) of this section. The content of benzathine penicillin G is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(d) *Total potency of a single-dose container.* Add sufficient distilled water to the material remaining in the 10-milliliter volumetric flask referred to in paragraph (b) of this section to bring the volume back to 10 milliliters and determine the number of units per milliliter of this suspension. If the iodometric method is used, 2.0-milliliter aliquots are placed in 50-milliliter volumetric flasks (one blank and one to be inactivated). Obtain the total potency by adding the number of units found in the 10-milliliter volumetric flask to one-half the content of buffered crystalline penicillin found in paragraph (b) of this section.

(e) *Sterility.* Proceed as directed in § 436.20 using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(f) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(g) *Pyrogens.* Proceed as directed in § 436.500.

(h) *Toxicity.* Proceed as directed in § 440.55a(b)(3) of this chapter.

(i) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the

product is reconstituted as directed in the labeling.

§ 436.507 Benzathine - procaine - buffered crystalline penicillins for aqueous injection.

(a) *Potency*—(1) *Total potency*. Proceed as directed in § 440.80a(b)(1) of this chapter, except if the bioassay method is used prepare the sample by diluting one dose of the drug suspension with sufficient dimethyl formamide or formamide or methyl alcohol to dissolve the benzathine penicillin G. Make to 100 milliliters with 1-percent phosphate buffer, pH 6.0. Shake well, and dilute to 1.0 unit per milliliter with buffer. If the iodometric method of assay is used, add the indicated amount of distilled water to the contents of a vial of the sample, shake well, and proceed as follows (except for single-dose containers):

(i) Using a standardized hypodermic syringe, withdraw one dose and dilute with 1-percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. Use 2.0 milliliters of this suspension as the blank in the iodometric assay procedure described in § 440.80a(b)(5)(iv)(*a*) of this chapter.

(ii) Using a standardized hypodermic syringe, withdraw another dose, place in a flask, and add 20 milliliters of 0.5 *N* NaOH for each 300,000 units of benzathine penicillin, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2 *N* HCl for each 2 milliliters of 0.5 *N* NaOH, mix, and dilute with distilled water to the same volume as was used in paragraph (a)(1)(i) of this section. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and titrate with 0.01 *N* sodium thiosulfate as directed in the iodometric assay procedure in § 440.80a (b)(5)(iv)(*a*) of this chapter. The total potency of the batch is satisfactory if it contains not less than 85 percent of that which it is represented to contain.

(2) *Procaine penicillin content* (*except for single-dose containers*). Make suitable dilutions of the solution prepared in paragraph (a)(1)(ii) of this section to obtain approximately 60 units of procaine penicillin per milliliter. Determine the procaine penicillin content by the colorimetric procedure described in § 436.503(b)(3). The content of procaine penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(3) *Buffered crystalline penicillin content*—(i) *Preparation of the solution for assay*. (*a*) Add the indicated amount of distilled water to the contents of a vial of the sample, and shake well. Withdraw one dose of the suspension with a hypodermic syringe and place in a 10-milliliter volumetric flask. Add 20-percent sodium sulfate solution almost to the mark, centrifuge sufficiently to see the meniscus, make to volume with 20-percent sodium sulfate solution, shake well, and centrifuge to obtain a clear or reasonably clear solution; or

(*b*) If the original product contains more than 600,000 units, place it in a 50-milliliter volumetric flask, add 20-percent sodium sulfate to the mark, shake well, place a 10-milliliter portion in a centrifuge tube, and centrifuge to obtain a reasonably clear solution.

(*c*) Dilute a 5.0-milliliter aliquot of the clear solution obtained in paragraph (a) (3)(i) (*a*) or (*b*) of this section with 1-percent phosphate buffer, pH 6.0, to give a solution for assay of approximately 2,000 units per milliliter.

(ii) *Iodometric assay for total penicillin in the solution for assay*. Determine the total quantity of penicillin in the solution for assay by the iodometric assay procedure described in § 440.80a (b)(5)(iv)(*a*) of this chapter.

(iii) *Colorimetric determination of procaine penicillin in the solution for assay*. Proceed as directed in § 436.503 (b)(3). The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(iv) The buffered crystalline penicillin in one dose of the product is calculated as follows:

$$A=(B-C)F,$$

where:

A=the buffered crystalline penicillin content of the product.

B=the number of units of penicillin per milliliter as determined in paragraph (a)(3)(ii) of this section.

C=the number of units of procaine penicillin per milliliter as determined in paragraph (a)(3)(iii) of this section.

F=the appropriate dilution factor depending on the dilutions made in the preparation of the solution for assay.

The content of buffered crystalline penicillin is satisfactory if the batch contains 85 percent of the number of units per milliliter that it is represented to contain.

(4) *Benzathine penicillin content.* The sum of the procaine penicillin content determined under paragraph (a)(2) or (6) of this section and the buffered crystalline penicillin content determined under paragraph (a)(3) of this section, subtracted from the total potency determined in paragraph (a)(1) or (5) of this section, represents the benzathine penicillin G content. The benzathine penicillin G content is satisfactory if it is not less than 85 percent of the number of units that it is represented to contain.

(5) *Total potency of a single-dose container.* Wash out the material remaining in the volumetric flask referred to in paragraph (a)(3)(i)(*a*) of this section, or combine the contents remaining in the 50-milliliter volumetric flask and in the centrifuge tube referred to in paragraph (a)(3)(i)(*b*) of this section. Dissolve the material by adding 10 milliliters of 1 *N* NaOH for each 300,000 units of benzathine penicillin and allow to stand 15 minutes. Add 1 milliliter of 1.2 *N* HCl for each milliliter of 1 *N* NaOH and then dilute with distilled water to give a concentration of approximately 2,000 units per milliliter. Place 2.0 milliliters in a 125-milliliter glass-stoppered Erlenmeyer flask, add 10 milliliters of 0.01 *N* iodine, allow to stand for 15 minutes, and then titrate with 0.01 *N* sodium thiosulfate as directed in § 440.80a (b)(5)(iv)(*a*) of this chapter. For the blank determination prepare a separate sample as directed in paragraph (a)(3)(i) (*a*) or (*b*) of this section and in the first sentence of this paragraph (a)(5), then dilute with 1 percent phosphate buffer, pH 6.0, to give a concentration of approximately 2,000 units per milliliter. The total potency of the one-dose container is equal to the sum of the number of units found in this assay (units per milliliter×volume) and the number of units found (units per milliliter ×volume) in the solution for assay in paragraph (a)(3)(ii) of this section.

(6) *Procaine penicillin content of a single-dose container.* Make suitable dilutions of the NaOH-inactivated solution prepared in paragraph (a)(5) of this section to obtain approximately 60 units of procaine penicillin per milliliter. Determine the procaine penicillin content (units per milliliter× volume) of this solution by the colorimetric procedure described under § 436.503(b)(3). To this value add per procaine penicillin content (unit per milliliter×volume) of the solution for assay, as found in paragraph (a)(3)(iii) of this section, to obtain the procaine penicillin content of the one-dose container. The content of procaine penicillin in the batch is satisfactory if it is not less than 85 percent of that which it is represented to contain.

(b) *Sterility.* Proceed as directed in § 436.20, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation, shake the tubes at least once daily.

(c) *Pyrogens.* Proceed as directed in § 440.55a(b)(4) of this chapter.

(d) *Toxicity.* Proceed as directed in § 440.55a(b)(3) of this chapter.

(e) *Moisture.* Proceed as directed in § 440.74a(b)(5) of this chapter.

(f) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the suspension resulting when the product is reconstituted as directed in the labeling.

§ 436.508 Penicillin - bacitracin - neomycin ointment; penicillin-bacitracin-neomycin in oil.

(a) *Potency*—(1) *Penicillin content; bacitracin content.* Proceed as directed in § 436.504(a).

(2) *Neomycin content.* Proceed as directed in § 448.510d(b)(1)(ii) of this chapter, except that sufficient penicillinase is added to the sample under test to completely inactivate the penicillin present. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

§ 436.509 Procaine penicillin-streptomycin-polymyxin in oil; procaine penicillin-dihydrostreptomycin-polymyxin in oil; procaine penicillin-streptomycin-polymyxin ointment; procaine penicillin - dihydrostreptomycin - polymyxin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 544.373(b)(1)(i) of this chapter, except inactivate the penicillin in the combined extractives with sufficient penicillinase at 37° C. for 30 minutes. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as a standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams per milliliter or per gram that it is represented to contain.

(4) *Polymyxin content.* Proceed as directed in § 444.170a(b)(2)(i) of this chapter, with the following exceptions:

(i) In lieu of the directions for the preparation of the sample described in § 444.170a(b)(2)(i)(*g*) of this chapter, prepare the sample by one of the following techniques:

(*a*) *Extraction.* Place a convenient-sized representative quantity of the sample in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer (pH 6.0) and shake. Remove the buffer layer and repeat the extraction with 25-milliliter portions of buffer at least three times and any additional times that may be necessary to insure complete extraction of the antibiotic. Combine the extractives. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes. Make the proper estimated dilutions in 10-percent potassium phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(*b*) *Blending.* Place a convenient-sized representative quantity of the sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 1-percent phosphate buffer (pH 6.0) to give a final volume of 200 milliliters. If the sample consists of substantially more than 1 gram, use sufficient buffer to give a final volume of 500 milliliters. If the concentration of polymyxin in the blend is less than 200 units per milliliter, 10-percent phosphate buffer (pH 6.0) should be used in lieu of 1-percent phosphate buffer (pH 6.0). Using a high-speed blender, blend the mixture for 2 minutes. Inactivate the penicillin with sufficient penicillinase at 37° C. for 30 minutes and make the proper estimated dilutions in 10-percent phosphate buffer (pH 6.0) to give a concentration of 10 units per milliliter (estimated).

(ii) The standard curve is prepared in the following concentrations: 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter in 10-percent potassium phosphate buffer, pH 6.0. The 10 units per milliliter concentration is used as the reference point. Its content of polymyxin is satisfactory if it contains not less than 85 percent of the number of units per milliliter or per gram that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975; 41 FR 10886, Mar. 15, 1976]

§ 436.510 Penicillin-streptomycin-erythromycin ointment; penicillin-dihydro-streptomycin-erythromycin ointment.

(a) *Potency*—(1) *Penicillin content.* Obtain the weight of the content of a syringe by weighing before and after ejecting the content into a beaker. Stir until homogeneous. Remove a representative sample (usually approximately 1.0 gram, accurately weighed) and place in a separatory funnel containing 50 milliliters of peroxide-free

ether. Add 20 milliliters of 0.1 *M* potassium phosphate buffer (pH 8.0) and shake. Remove the buffer layer and repeat the extraction with three additional 20-milliliter portions of the buffer. Place the buffer solution in a second separatory funnel and wash with three 30-milliliter portions of ether. Discard the ether washes. Remove an aliquot of the buffer solution and proceed as directed in § 440.80a(b) (1) of this chapter, except § 440.80a(b)(1)(iv) and (ix) of this chapter. If the iodometric chemical assay is used, proceed as directed in § 440.80a(b)(5)(iv)(*a*) of this chapter, except prepare the sample as directed in § 536.501(a)(1) of this chapter. Its content of penicillin is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Using an aliquot of the buffer solution prepared as directed in paragraph (a)(1) of this section, proceed as directed in § 444.70a (b)(1) through (9) of this chapter, except add sufficient penicillinase to completely inactivate the penicillin present. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in paragraph (a)(2) of this section, using the dihydrostreptomycin working standard as the standard of comparison. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Erythromycin content.* Proceed as directed in § 444.570b(b)(1)(i)(*b*) of this chapter, except prepare the sample as follows: Place a representative sample (usually approximately 1.0 gram, accurately weighed), in a glass blending jar containing 99 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and 1 milliliter of polysorbate 80. Using a high-speed blender, blend for 2 to 3 minutes. Add 100 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0, and blend for an additional 2 to 3 minutes. Prepare an intermediate dilution by diluting an aliquot of the filtrate with 0.1 *M* potassium phosphate buffer (pH 8.0), and add sufficient penicillinase to inactivate the penicillin. Then further dilute with buffer to give an erythromycin content of 1.0 microgram per milliliter (estimated). Its content of erythromycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is presented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.511 Penicillin-streptomycin-bacitracin methylene disalicylate-neomycin ointment; penicillin-dihydrostreptomycin-bacitracin methylene disalicylate-neomycin ointment.

(a) *Potency*—(1) *Penicillin content.* Proceed as directed in § 540.380a(b)(1) of this chapter. Its penicillin content is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Streptomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of streptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Dihydrostreptomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Bacitracin methylene disalicylate content.* Proceed as directed in § 436.505(a)(3). Its potency is satisfactory if it contains not less than 85 percent of the equivalent number of units of bacitracin that it is represented to contain.

(5) *Neomycin content.* Proceed as directed in § 436.105 of this chapter. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.201.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.512 Procaine penicillin G-novobiocin-neomycin-dihydrostreptomycin in oil.

(a) *Potency*—(1) *Penicillin G content.* Proceed as directed in § 440.180d (b)(1)(i)(*a*) of this chapter, using the

novobiocin-resistant strain of *Staphylococcus aureus* (ATCC 12692),[1] except prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 1 percent potassium phosphate buffer, pH 6.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender and make appropriate dilutions, using 1 percent potassium phosphate buffer, pH 6.0. Its content of penicillin G is satisfactory if it contains not less than 85 percent of the number of units that it is represented to contain.

(2) *Novobiocin content.* Proceed as directed in § 440.180d(b)(3)(i), with the following exceptions:

(i) Prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6) to give a final concentration of 0.5 microgram novobiocin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 50 WX–4, Na^+ type 200–400 mesh, sufficient to make a total concentration of 2 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of novobiocin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(3) *Neomycin content.* Proceed as directed in § 436.517(b)(1) of this chapter, using the *Staphylococcus epidermidis* (ATCC 12228)[1] procedure, except:

(i) Prepare the sample as follows: Place the equivalent of one dose of sample in a blending jar, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram neomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates.

(ii) Aseptically add to the seed agar used for this assay, at the time the bacterial suspension is added, a slurry of Dowex 1–X8, Cl type 200–400 mesh, to make a total concentration of 1 percent. Prepare the slurry by adding 50 grams of the resin to 30 milliliters of distilled water and sterilize for 15 minutes at 15 pounds pressure. Mix the slurry thoroughly before adding. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(4) *Dihydrostreptomycin content.* Proceed as directed in § 436.105 except prepare the sample by placing the equivalent of one dose in a blender, add 1.0 milliliter of polysorbate 80 and a quantity of 0.1*M* potassium phosphate buffer, pH 8.0, sufficient to make a total of 500 milliliters. Blend for 5 minutes with a high-speed blender. To an aliquot, add sufficient penicillinase to inactivate the penicillin, further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram dihydrostreptomycin per milliliter (estimated), and allow to stand for ½-hour at 37° C. before filling the plates. Its content of dihydrostreptomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 436.500(c).

[39 FR 18944, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976]

§ 436.513 Chlortetracycline troches; tetracycline hydrochloride troches.

(a) *Potency.* If it is tetracycline hydrochloride proceed as directed in § 446.81a(b)(1) of this chapter and if it is

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

chlortetracycline hydrochloride troches proceed as directed in § 446.10a(b)(1) of this chapter, except § 446.10a(b)(1)(x), and in lieu of the directions in § 446.10a(b)(1)(iv) and (viii)(*c*) of this chapter prepare the sample as follows: Place 12 troches in a glass blending jar containing 500 milliliters of 0.01*N* HCl. Using a high-speed blender, blend for 3 to 5 minutes and then make the proper estimated dilutions in the buffer solution. The average potency of the troches is satisfactory if they contain not less than 85 percent of the number of milligrams they are represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

§ 436.514 Chlortetracycline hydrochloride powder topical; tetracycline hydrochloride powder topical.

(a) *Potency*—(1) *Dry powder.* Using a 3.0-gram sample or the entire contents of the immediate container for each determination, prepare the sample as follows: Using a high-speed blender, blend a 3.0-gram sample in a glass blending jar containing 500 milliliters of 0.01 *N* HCl (use 0.1 *N* HCl if it is tetracycline), or reconstitute in the immediate container as directed in the labeling of the drug. Transfer an appropriate aliquot of 1.0 milliliter to 5.0 milliliters to a 100-milliliter volumetric flask and make to mark with 0.01 *N* HCl (use 0.1 *N* HCl if it is tetracycline). Withdraw an aliquot from the volumetric flask, and if it is chlortetracycline hydrochloride dilute to 0.06 μ g. per milliliter, using 0.1 *M* potassium phosphate buffer, pH 4.5, and proceed as directed in § 446.10a(b)(1) of this chapter. If it is tetracycline hydrochloride, dilute to 0.24 μ g. per milliliter, using 0.1 *M* potassium phosphate buffer, pH 4.5, and proceed as directed in § 446.81a(b)(1) of this chapter. The average potency is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline hydrochloride or tetracycline hydrocloride per gram or per immediate container that it is represented to contain.

(2) *Powder packaged with inert gases.* Spray, as directed in the labeling, the entire contents of each container to be tested into a separate 2-liter Erlenmeyer flask, held in a horizontal position. Add 500 milliliters of 0.1 *N* HCl and shake to dissolve the contents. Immediately remove aliquots of this solution and, using 0.1 *M* potassium phosphate buffer, pH 4.5, for further dilutions, proceed as directed in § 446.10a(b)(1) of this chapter if it is chlortetracycline hydrochloride powder or § 446.81a(b)(1) of this chapter if it is tetracycline hydrochloride powder. Calculate the average total amount of antibiotic expelled from the containers. The total potency is satisfactory if it contains not less than 85 percent of the number of milligrams of chlortetracycline hydrochloride or tetracycline hydrochloride that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter, except if it is packaged with inert gases proceed as directed in § 536.513(c) of this chapter.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.515 Capsules tetracycline and oleandomycin phosphate; capsules tetracycline and troleandomycin; capsules tetracycline hydrochloride and oleandomycin phosphate; capsules tetracycline hydrochloride and troleandomycin.

(a) *Potency*—(1) *Tetracycline or tetracycline hydrochloride content by turbidimetric assay*—(i) *Test culture and media.* Maintain the test organism *Escherichia coli* (ATCC 10536)[1] on the agar described in § 440.80a(b)(1) (ii)(*a*) of this chapter. For use in the assay, prepare a suspension of the organism every 2 weeks, as follows: Transfer the organism to a fresh agar slant and incubate at 37°C. overnight. Wash the growth from the slant with the aid of 2 milliliters of sterile distilled water and sterile glass beads into a Roux bottle containing 300 milliliters of the maintenance medium. Incubate overnight at 37° C. and then harvest the growth with 50 milliliters of sterile distilled water and sterile glass beads. Standardize this suspension by determining the dilution that will permit 40-percent light

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

transmission in a photoelectric colorimeter using a 650-millimicron filter and an 18-millimeter diameter test tube as an absorption cell. Prepare the daily inoculum by adding 10 milliliters of that dilution to each liter of nutrient broth, prepared as directed in § 440.80a (b)(1)(ii)(*c*) of this chapter, needed for the test.

(ii) *Working standard and solutions.* Dissolve an appropriate amount of the working standard in sufficient 0.1 *N* HCl to give a concentration of 1,000 micrograms per milliliter. This stock solution may be kept in the refrigerator for 1 week. Make daily dilutions of the stock solution with 0.1 *M* potassium phosphate buffer (pH 4.5) to obtain concentrations of 0.146, 0.187, 0.240, 0.308, and 0.395 micrograms per milliliter. Add 1.0 milliliter of each such concentration to each of three 16 millimeters x 125 millimeters test tubes.

(iii) *Preparation of sample.* Dissolve the contents of a representative number of capsules in sufficient 0.1 *N* HCl to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1 *M* potassium phosphate buffer (pH 4.5) to obtain a final concentration of 0.24 microgram per milliliter (estimated). Add 1.0-milliliter of this dilution to each of three 16 millimeters x 125 millimeters test tubes.

(iv) *Procedure.* To each of the 16 millimeters x 125 millimeters test tubes prepared in paragraph (a)(1)(ii) and (iii) of this section, add 9.0 milliliters of the inoculated nutrient broth described in paragraph (a)(1)(i) of this section and place immediately in a 37° C. water bath for 3 to 4 hours. After incubation, add 0.5 milliliter of a 12-percent formaldehyde solution to each tube and read the absorbance values in a suitable photoelectric colorimeter using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear uninoculated broth prepared as described in § 440.80a(b)(1)(ii)(*c*) of this chapter.

(v) *Estimation of potency.* Plot the average values for each concentration of the standard on arithmetic graph paper with absorbance values on the ordinate and tetracycline or tetracycline hydrochloride concentrations on the abscissa. Construct the best straightline through the points, either by inspection or by means of the following equations:

$$L=(3a+2b+c-e)/5,$$
$$H=(3e+2d+c-a)/5,$$

where:

L=absorbance value for the lowest concentration of the standard curve.

H=absorbance value for the highest concentration of the standard curve.

a, b, c, d, e=average absorbance values for each concentration of the standard curve.

Plot the values obtained for *L* and *H* and connect the points with a straight line. Average the absorbance values for the sample and read the tetracycline or tetracycline hydrochloride concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the tetracycline or tetracycline hydrochloride content of the sample. Its potency is satisfactory if it contains the equivalent of not less than 85 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain.

(2) *Oleandomycin content.* (i) If oleandomycin phosphate is used, proceed as directed in paragraph (c)(1) of this section, except prepare the sample as follows: Dissolve the contents of a representative number of capsules in sufficient 0.1 *M* potassium phosphate buffer (pH 8.0) to give a stock solution of convenient concentration. Further dilute with 0.1 *M* potassium phosphate buffer (pH 8.0) to obtain a final concentration of 5.0 μ g. of oleandomycin activity per milliliter (estimated).

(ii) If troleandomycin is used, proceed as follows: Dissolve the contents of a representative number of capsules in chloroform to give a stock solution of 1.0 milligram of oleandomycin activity per milliliter. Transfer 30 milliliters of the chloroform solution to a glass-stoppered test tube (200 millimeters x 22 millimeters) and add 20 milliliters of 1 *N* sodium hydroxide. Shake for 1 minute and centrifuge briefly to aid in the separation of the layers. With the aid of a syringe and needle, remove and discard the aqueous layer. Repeat the washing procedure with two more 20-milliter portions of 1 *N* sodium hydroxide solution. Filter the chloroform layer through a pledget of cotton.

Dilute an aliquot of this solution with chloroform to give a solution containing approximately 25 μ g. of oleandomycin per milliliter. Transfer a 5.0 milliliter aliquot to a 40 milliliter glass-stoppered centrifuge tube, dilute to 20 milliliters, with chloroform, and determine the oleandomycin content as directed in paragraph (d)(1)(i) of this section.

Its content of oleandomycin is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Moisture.* Proceed as directed in § 440.80a(b)(5)(1) of this chapter.

(c) *Oleandomycin phosphate used in making the capsules*—(1) *Potency*—(i) *Cylinders* (*cups*). Used cylinders described in § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture media.* (*a*) Use the nutrient agar described in § 440.80a (b)(1)(ii)(*a*) of this chapter for the seed layer and base layer, except that its pH after sterilization is 7.8 to 8.0.

(*b*) Use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter for maintaining the test organism.

(iii) *Working standard.* Dissolve a suitable weighed quantity (usually 25 milligrams or less) of the working standard (obtained from the Food and Drug Administration) in 2 milliliters of ethanol, then add sufficient 0.1 *M* potassium phosphate buffer, pH 8.0, to give a concentration of 1,000 micrograms of oleandomycin base per milliliter. This stock solution may be kept in the refrigerator for 3 days.

(iv) *Preparation of sample.* Dissolve the sample in sufficient 0.1 *M* potassium phosphate buffer (pH 8.0) to give a convenient stock solution. Further dilute in 0.1 *M* potassium phosphate buffer (pH 8.0) to give a final concentration of 5.0 micrograms per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC 12228)[1] which is maintained on slants or agar described under paragraph (c)(1)(ii)(*a*) of this section. Wash the organism from the agar slant with 3 milliliters of sterile physiological saline solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in paragraph (c)(1)(ii)(*a*) of this section. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 4 hours at 32° C. and then wash the resulting growth from the agar surface with about 30 milliliters of sterile physiological saline solution. Standardize the suspension by determining the dilution that will give 80-percent light transmission, using a suitable photoelectric colorimeter with a 650-millimicron filter and an 18-millimeter-diameter test tube as an absorption cell. Run test plates to determine the quantity of the diluted suspension (usually 1.5 milliliters) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (c)(1)(ii)(*a*) of this section to each Petri dish (20 millimeters × 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in paragraph (c)(1)(ii)(*a*) of this section, cool to 48° C., add the proper amount of the test organism as described in paragraph (c)(1)(v) of this section and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1 *M* potassium phosphate buffer (pH 8.0) to obtain concentrations of 3.2, 4.0, 5.0, 6.25 and 7.80 micrograms per milliliter. Use 3 plates for the determination of each point on the curve, except the 5.0 micrograms per milliliter concentration, a total of 12 plates. On each of 3 plates fill 3 cylinders with the 5.0 micrograms per milliliter standard, and the other 3 cylinders with the concentration under test. Thus, there

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

will be 36 five-microgram determinations and 9 determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the point tested for each set of 3 plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of 3 plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0-microgram concentration were 20.0 millimeters, and the average of the 5.0-microgram concentration of this set of 3 plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 4.0-microgram concentration of these same 3 plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=(3a+2b+c-e)/5,$$
$$H=(3e+2d+c-a)/5,$$

where:

L=corrected zone diameter for the lowest concentration of the standard curve,

H=corrected zone diameter for the highest concentration of the standard curve,

c=average zone diameter for 36 readings of the 5.0 micrograms per milliliter standard.

a, b, d, e=corrected average values for the 3.2, 4.0, 6.25, and 7.81 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay*. Use 3 plates for each sample. Fill 3 cylinders on each plate with the standard 5.0 micrograms per milliliter solution and 3 cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the 3 plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 5.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 5.0 micrograms per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity*. Proceed as directed in § 440.80a(b)(4) of this chapter, except use physiological salt solution as the diluent, and inject 0.5 milliliter of a solution containing 8 milligrams per milliliter.

(3) *Moisture*. Proceed as directed in § 440.80(b)(5)(i) of this chapter.

(4) *pH*. Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 100 milligrams per milliliter.

(5) *Crystallinity*. Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

(d) *Troleandomycin used in making the capsules*—(1) *Potency*—(i) *Chemical method*—(*a*) *Reagents and equipment*. (*1*) Methyl orange reagent: Shake 0.5 *M* boric acid solution for about 12 hours (to insure saturation) with an excess of methyl orange indicators. An alternative method is to heat the mixture to about 50° C. and shake for about an hour. Then allow to cool. Filter the saturated dye solution and wash three times with chloroform. Store the dye solution over chloroform.

(*2*) Acid-alcohol solution: Add 2 milliliters of concentrated sulfuric acid to 98 milliliters of absolute methyl alcohol.

(*3*) Glycerin: Reagent grade.

(*4*) Centrifuge tubes: 40 milliliters, glass-stoppered.

(*b*) *Procedure.* Prepare a chloroform solution containing 50.0 milligrams activity of standard oleandomycin base in 200 milliliters of solution. Transfer 10.0 milliliters of the solution to a 100-milliliter volumetric flask and dilute to volume with chloroform. Transfer 2.0, 4.0, 6.0, and 8.0 milliliters of this solution to glass-stoppered centrifuge tubes (40-milliliter size) and dilute to a total volume of 20.0 milliliters each with chloroform. To the 20.0 milliliters of the solution present in each (40-milliliter size) centrifuge tube add 0.2 milliliter of glacial acetic acid, 0.20 milliliter of glycerin, and 0.40 milliliter of methyl orange reagent. Shake for 5 minutes and centrifuge for 3 minutes. Immediately transfer to another tube a 10.0-milliliter aliquot from the chloroform (lower) layer. Care must be exercised to see that no portion of the dye-glycerin-phase is included with the chloroform aliquot. Add 1.0 milliliter of acid-alcohol solution to this chloroform aliquot, mix well, and read the absorbancy at 535 mμ, using a 1-centimeter cell and a suitable photometer and using chloroform, similarly treated, as a blank. Prepare a standard curve, plotting the absorbance values of the standard solutions against the concentration expressed in micrograms per aliquot. Accurately weigh the sample to be tested to give 50 milligrams (estimated) of oleandomycin activity, dissolve in chloroform, and make to 200 milliliters with chloroform. Transfer 10.0 milliliters to a 100-milliliter volumetric flask and make to volume with chloroform. Transfer 5.0 milliliters to a glass-stoppered centrifuge tube and proceed as above. Determine the potency of the sample from the standard curve.

(ii) *Microbiological assay.* Proceed as directed in paragraph (c)(1) of this section, except:

(*a*) In lieu of the directions in paragraph (c)(1)(ii)(*a*) of this section, use the nutrient agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter for the seed and base layers, except add 2.0 milliliters of polysorbate 80 to each 100 milliliters of agar. Its pH after sterilization is 7.8 to 8.0.

(*b*) In lieu of the directions in paragraph (c)(1)(iii) of this section, dissolve a suitable weighed quantity (usually 25 milligrams or less) of the troleandomycin working standard (obtained from the Food and Drug Administration) in sufficient 80 percent isopropyl alcohol-water solution to give a concentration of 1,000 micrograms per milliliter (estimated). Use the solution the day that it is prepared.

(*c*) In lieu of the directions in paragraph (c)(1)(iv) of this section, dissolve the sample in sufficient 80 percent isopropyl alcohol-water solution to give a convenient stock solution. Further dilute in 0.2 *M* potassium phosphate buffer, pH 10.5 (35 grams of dipotassium phosphate plus 2 milliliters of 10 *N* NaOH, q.s. to 1 liter), to give a final concentration of 15 micrograms per milliliter (estimated).

(*d*) In lieu of the directions in paragraph (c)(1)(vi) of this section, use the agar described in paragraph (d)(1)(ii)(*a*) of this section for both layers. Use the plates as soon after seeding as is practical. If they are not to be used shortly after seeding, then they should be refrigerated until ready for use.

(*e*) In lieu of the directions for preparing the standard curve in paragraph (c)(1)(vii) of this section, prepare the standard curve by diluting the stock solution in 0.2 *M* potassium phosphate buffer, pH 10.5, to give concentrations of 9.6, 12.0, 15.0, 18.8, and 23.4 micrograms per milliliter. The 15.0 micrograms per milliliter is the reference concentration.

(*f*) In lieu of the directions in paragraph (c)(1)(viii) of this section, incubate the plates at 37° C. overnight. The concentration of the sample and standard being tested is 15.0 micrograms per milliliter.

(2) *Toxicity.* Administer orally, by means of a cannula or other suitable device, to each of five mice within the weight range of 18 grams to 25 grams, 0.5 milliliter of a suspension containing 200 milligrams per milliliter in normal saline solution. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test, using for each test five or more previously unused mice weighing 20 grams (±0.5 gram) each; if the total deaths within 48 hours is no greater than 10 percent of

the total number of animals tested, including the original test, the sample is nontoxic.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a saturated aqueous-ethanol (1:1) solution prepared by adding 100 milligrams per milliliter.

(5) *Paper chromatograph method*—(i) *Apparatus and reagents*—(*a*) Chromatographic chamber (cylinder glass-stoppered museum jar 11.5 inches × 3.5 inches).

(*b*) Chromatographic paper (8 inches × 8 inches Whatman No. 1).

(*c*) 0.1 *N* hydrochloric acid.

(*d*) Resolving solvent: Butyl acetate, benzene, nitromethane, pyridine (5:5:5:1 by volume).

(*e*) Spray reagent: 15 grams antimony trichloride per 100 milliliters of chloroform.

(ii) *Procedure.* Dissolve the sample in chloroform to give a solution containing 10 milligrams to 20 milligrams per milliliter. Prepare a sheet of chromatographic paper by drawing a line of origin parallel to and 1 inch from the edge of the paper. Wet the paper thoroughly with the 0.1 *N* hydrochloric acid and blot it firmly between sheets of absorbent paper. Starting 2 inches in from the edges and at 1-inch intervals, apply 3 microliters to 5 microliters of the sample solutions to the starting line. Allow a few minutes for the paper to dry partially. While the paper is still damp, form a cylinder by bringing the outer edges together, allowing about 1-inch overlap, and secure with a paper clip. Stand the paper in the chromatographic chamber, which has been filled to a depth of ½-inch with the resolving solvent. After the solvent front rises to a height of 4 inches to 5 inches above the origin, remove the paper from the tank and hang it up to air dry. Spray the dried paper with the antimony trichloride reagent. Hang the paper in a 100° C. oven for 3 minutes. A purple spot becomes visible for trioleandomycin at an R_f value of about 0.85. The approximate R_f values for diacetyloleandomycin, monoacetyloleandomycin, and oleandomycin are, respectively, 0.72, 0.27, and 0.13.

(6) *Acetyl determination*—(i) *Apparatus and reagents.* (*a*) One three-necked Pyrex flask of approximately 45 milliliters capacity, pear-shaped with T-joints, agar inlet tube, glass-stoppered funnel, glass condenser, and bubble counter.

(*b*) 50-milliliter Pyrex Erlenmeyer flask.

(*c*) 10-milliliter burette, calibrated in 0.02 milliliter.

(*d*) Anhydrous methanol, reagent grade.

(*e*) 2 *N* sodium hydroxide solution.

(*f*) Sulfuric acid solution prepared by adding 100 milliliters of concentrated H_2SO_4 to 200 milliliters of water.

(*g*) 1 *N* barium chloride solution.

(*h*) Phenolphthalein solution (1 percent in ethanol).

(*i*) Water-pumped nitrogen.

(*j*) NaOH solution, 0.015 *N*.

(ii) *Procedure.* Weight accurately (to 0.01 milligram) approximately 30 milligrams of the sample into the three-necked acetyl flask. Add 2.0 milliliters of methanol to dissolve the sample, then add slowly with gentle swirling, 1.0 milliliter of NaOH solution. Connect the gas inlet tube with bubble counter attached, and adjust nitrogen flow to about two bubbles a second. Put glass-stoppered funnel in centerneck of acetyl flask and put about 5 milliliters of H_2O in the funnel. Add a boiling chip to the solution and attach condenser in the refluxing position with water cooling. Adjust burner flame under acetyl flask to reflux solution gently. Reflux for 30 minutes. Cool assembly slightly then rinse down condenser (still in reflux position) with a few milliliters of H_2O. Reassemble condenser to the distillation position and add water through the funnel to make a total of approximately 5 milliliters of H_2O added to acetyl flask. Adjust burner flame so that about 5 milliliters of H_2O and methanol is distilled over in approximately 10 minutes. Discard this distillate. Cool acetyl flask slightly. Acidify solution in flask by adding 1 milliliter of the sulfuric acid solution through the funnel. Adjust burner flame and distill over approximately 20

milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnel as necessary. It is important to keep the liquid volume in the acetyl flask around 2 milliliters to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first reaction and boil gently about 20 seconds. Add a few drops of $BaCL_2$ solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent immediately titrate the solution with the 0.015 *N* NaOH solution to a faint pink endpoint, using one drop of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015 *N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

(Milliliters of NaOH × *N* NaOH × 0.043 × 100)/ Weight sample in grams=Percent acetyl.

(7) *Crystallinity*. Proceed as directed in § 440.80a(b)(5)(iii) of this chapter.

§ 436.516 Tetracycline-neomycin complex powder topical; tetracycline hydrochloride-neomycin sulfate powder topical.

(a) *Potency*—(1) *Tetracycline-neomycin complex powder*—(i) *Tetracycline content*. Proceed as directed in § 436.514(a)(2), except use water in lieu of 0.1 *N* HCl for dissolving the sample. Its tetracycline content is satisfactory if it contains not less than 85 percent of the equivalent number of milligrams of tetracycline hydrochloride that it is represented to contain.

(ii) *Neomycin content*. Using 0.1 *M* potassium phosphate buffer, pH 8.0, dilute an appropriate aliquot of the aqueous solution, prepared as directed in paragraph (a)(1) of this section, to a final concentration of 1 μ g. per milliliter (estimated), and proceed as directed in § 436.515(c)(1), except that the neomycin standard stock solution described § 436.517(b)(1)(iii) is used to prepare the standard curve, by further diluting with pH 8.0 buffer to final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 μ g. per milliliter. The 1.0 μ g per milliliter solution is the reference concentration. In lieu of the method described in this subparagraph, the neomycin content may also be determined as follows. Using the aqueous solution described, prepare the sample and proceed as directed in § 436.517(b)(1), except use *Staphylococcus aureus* (American Type Culture Collection 12715)[1] as the test organism, which is grown and maintained on agar containing 100 μ g. of tetracycline hydrochloride per milliliter of agar. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(2) *Tetracycline hydrochloride-neomycin sulfate powder*—(i) *Tetracycline hydrochloride content*. Prepare the sample as directed in § 436.514(a)(2). Use an appropriate aliquot and proceed as directed in § 446.81a(b)(1) of this chapter. Its tetracycline hydrochloride content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(ii) *Neomycin content*. Use an appropriate aliquot of the solution prepared in paragraph (a)(2)(i) of this section and proceed as directed in paragraph (a)(1)(ii) of this section. Its neomycin content is satisfactory if it contains not less than 85 percent of the number of milligrams that it is represented to contain.

(b) *Sterility*. Thoroughly cleanse with a suitable disinfectant the value (do not flame) of each container to be tested. Into each of two empty, sterile Erlenmeyer flasks stoppered with a cotton plug, spray quantitites sufficient to yield a residue of approximately the equivalent of 50 milligrams from 10 separate cans by removing the plug temporarily and using aseptic technique while spraying; allow propellant to evaporate, add 250 milliliters to 500 milliliters of diluting fluid B in lieu of diluting fluid A, and swirl the flasks to dissolve the contents. Then proceed as

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

directed in § 436.20 of this chapter using the method described in paragraph (e)(1) of that section.

(c) *Moisture.* Proceed as directed in § 536.513(c) of this chapter.

(d) *Tetracycline-neomycin complex used in making the drug*—(1) *Potency*—(i) *Tetracycline content.* Dissolve the sample to be tested in sufficient water to give a convenient stock solution. Using an appropriate aliquot, proceed as directed in § 446.81a(b)(1).

(ii) *Neomycin content.* Using an aliquot of the stock solution prepared as directed in paragraph (d)(1)(i) of this paragraph, proceed as directed in paragraph (a)(2) of this section, except the last sentence of that subparagraph.

(2) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter using 0.5 milliliter of a solution prepared by diluting the sample with physiological sodium chloride solution to contain 200 μ g. of neomycin per milliliter (estimated).

(3) *pH.* Using a 1-percent aqueous solution, proceed as directed in § 440.80a(b)(5)(ii) of this chapter.

[39 FR 18944, May 30, 1974, as amended at 40 FR 13497, Mar. 27, 1975]

§ 436.517 Bacitracin-neomycin tablets; zinc bacitracin-neomycin tablets; bacitracin methylene disalicylate-neomycin tablets.

(a) *Tablets*—(1) *Potency*—(i) *Bacitracin, zinc bacitracin, or bacitracin methylene disalicylate content.* Proceed as directed in § 448.110a(b)(1). Its content of bacitracin, zinc bacitracin, or bacitracin methylene disalicylate is satisfactory if it contains not less than 85 percent of the number of units per tablet that it is represented to contain.

(ii) *Neomycin content.* Place 5 tablets in a blending jar and add thereto 200 milliliters of a 500-milliliter quantity of 0.10-percent phosphate buffer pH 8.0. After blending for 1 minute with a high-speed blender, add the remainder of the buffer. Blend again for 1 minute and make the proper estimated dilutions in the buffer and proceed as directed in paragraph (b)(1) of this section. Its content of neomycin is satisfactory if it contains not less than 85 percent of the number of milligrams of activity that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3).

(b) *Neomycin used in making the tablets*—(1) *Potency*—(i) *Cylinders* (*cups*). Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture medium.* Use the medium described in § 440.80a(b)(1) (ii)(*a*) of this chapter for both the base and seed layers, except its pH after sterilization is 7.8 to 8.0.

(iii) *Working standard.* Dry the working standard (obtained from the U.S.P. Reference Standards Committee, 46 Park Avenue, New York 16, N.Y.) for 3 hours at 60° C. and a pressure of 5 millimeters or less and weigh out a sufficient quantity to make a convenient stock solution by diluting with a 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0. The stock solution, when stored at a temperature of approximately 15° C., or less, may be used for a period not exceeding 1 month.

(iv) *Standard curve.* Using the stock solution, prepare a daily standard curve as directed in § 444.70a(b)(1)(iv) of this chapter, using solutions of the neomycin working standard in 0.1*M* potassium phosphate buffer, pH 8.0, in concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 micrograms per milliliter if the test organism *Staphylococcus aureus* (ATCC 6538P),[1] or in concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter if the test organism is *Staphylococcus epidermidis* (ATCC 12228).[1] The 10.0 micrograms per milliliter and the 1.0 microgram per milliliter concentrations are used as the reference points.

(v) *Preparation of test organism.* The test organism is *Staphylococcus aureus* (ATCC 6538P),[1] which is maintained on agar described in § 440.80a(b)(1)(ii)(*a*) of this chapter. From a stock slant inoculate a Roux bottle containing this same agar and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this suspension by determining the dilution that will permit 80 percent light transmission through a filter at 6500 Angstrom units

[1] See footnote 1 to § 436.516.

in a photoelectric colorimeter. The suspension may be used for 2 weeks if it is stored under refrigeration.*Staphylococcus epidermidis* (ATCC 12228),[1] which is maintained on agar as described in § 440.80a(b)(1)(ii)(*a*) of this chapter, may also be used as the test organism. From a stock slant, inoculate a Roux bottle containing this medium and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface, using approximately 30 milliliters of sterile sodium chloride solution. Standardize the suspension by determining the dilution that will permit 80 percent light transmission through a filter of 6500 Angstrom units in a photoelectric colorimeter. The suspension may be stored for 2 weeks under refrigeration.

(vi) *Preparation of plates.* Using the agar described in subdivision (ii) of this subparagraph and approximately a 0.5 percent inoculum of the suspension described in paragraph (b)(1)(v) of this section, prepare the plates as directed in § 440.80a(b)(1)(v) of this chapter.

(vii) *Assay.* Dissolve volumetrically in 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0, the sample to be tested to make a convenient stock solution. Further dilute volumetrically this solution with 0.1 *M* potassium phosphate buffer, pH 7.8 to 8.0, to a final concentration of 10.0 micrograms (estimated) per milliliter, if the test organism is *Staphylococcusaureus* or 1.0 microgram per milliliter (estimated) if the test organism is *Staphylococcus epidermidis.*

(2) *Toxicity.* Proceed as directed in § 440.80a(b)(4) of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 200 micrograms per milliliter with physiological salt solution.

(3) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with groundglass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 33 milligrams per milliliter.

§ 436.542 Acid resistance/dissolution test for enteric-coated erythromycin pellets.

(a) *Equipment.* Use Apparatus 1 as described in the United States Pharmacopeia XX dissolution test.

(b) *Immersion fluids.* All immersion fluids may be degassed by heating immediately prior to use.

(1) *Acid resistance medium.* Use 0.06*N* hydrochloric acid, pH 1.2.

(2) *Dissolution medium.* Dissolve 6.8 grams of monobasic potassium phosphate in 250 milliliters of water. Add 109 milliliters of 0.2*N* sodium hydroxide and 400 milliliters of water and adjust the resulting solution with 0.2*N* sodium hydroxide to a pH of 6.8±0.1. Dilute to 1 liter.

(c) *Procedure.* Warm the immersion fluids to a temperature of 37° ±5.0° C. Place the contents of one capsule into the basket. Lower the basket into 900 milliliters of acid resistance medium contained in the beaker. Ensure that all air is displaced from the immersed basket and that the pellets remain in the basket. Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 1 hour. Remove the basket from the fluid and immediately lower the basket into 900 milliliters of dissolution medium contained in the beaker. Again ensure that all air is displaced from the immersed basket and that the pellets remain in the basket. Rotate the basket at 50 revolutions per minute for an accurately timed dissolution period of 45 minutes. Withdraw a 25-milliliter sample of the dissolution medium from a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Filter the sample through a Whatman 541 filter paper or equivalent, discarding the first 2 milliliters. Assay for erythromycin using

the filtrate as the test solution as directed in § 436.105. Repeat the test on five additional capsules.

(d) *Evaluation.* Use the interpretation described in the United States Pharmacopeia XX dissolution test.

[46 FR 16678, Mar. 13, 1981, as amended at 50 FR 47213, Nov. 15, 1985; 52 FR 35912, Sept. 24, 1987; 54 FR 41824, Oct. 12, 1989]

§ 436.543 Acid resistance test for pellet-filled doxycycline hyclate capsules.

(a) *Equipment.* Use Apparatus 1 as described in the United States Pharmacopeia XXI dissolution test.

(b) *Acid resistance medium.* Use 0.06*N* hydrochloric acid, pH 1.2. May be degassed by heating immediately prior to use.

(c) *Procedure.* Warm the acid resistance medium to a temperature of 37±2.0 ° C. Place the contents of one pellet-filled capsule into the basket. Lower the basket into a beaker containing 900 milliliters of acid resistance medium. Ensure that all air is displaced from the immersed basket and that the contents of the pellet-filled capsule remain in the basket.

Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 20 minutes. Withdraw a 5-milliliter sample of the acid resistance medium from a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth (this is the sample solution). Assay the sample solution for doxycycline as described in paragraph (d) of this section. Repeat the test on five additional pellet-filled capsules.

(d) *Doxycycline content*—(1) *Preparation of working standard solution.* Dissolve an accurately weighed portion of doxycycline hyclate working standard with 0.1*M* hydrochloric acid to obtain a concentration of 0.01 milligram per milliliter.

(2) *Preparation of sample solution.* Dilute the 5-milliliter sample portion to 25 milliliters with 0.1*M* hydrochloric acid.

(3) *Procedure.* Using a suitable spectrophotometer and 0.1*M* hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 345 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(4) *Calculations.* Determine the total amount of doxycycline dissolved as follows:

$$\text{Milligrams of doxycycline dissolved} = \frac{A_u \times c \times d \times 900^*}{A_s}$$

where:

A_u=Absorbance of sample;

A_s=Absorbance of standard;

c=Concentration of working standard in milligrams; and

d=Dilution factor of sample withdrawn from beaker.

*If more than 15 milliliters of dissolution medium is removed, correct for the volume removed.

(e) *Evaluation.* The pellet-filled capsule passes the test if no more than 50 percent of the drug is dissolved at 20 minutes. If one pellet-filled capsule fails to meet this requirement, repeat the test on six additional pellet-filled capsules. No more than 2 pellet-filled capsules in 12 may exceed 50 percent of the drug dissolved at 20 minutes.

[50 FR 41679, Oct. 15, 1985; 50 FR 45603, Nov. 1, 1985]

§ 436.544 Dissolution test for pellet-filled doxycycline hyclate capsules.

(a) *Equipment.* Use Apparatus 1 as described in the United States Pharmacopeia XXI dissolution test.

(b) *Dissolution medium.* Prepare the dissolution medium as follows: Dissolve 10.21 grams of potassium biphthalate and 1.4 grams of sodium hydroxide in approximately 950 milliliters of distilled water and adjust the pH to 5.5 using 1*M* sodium hydroxide solution. Dilute with distilled water to 1,000 milliliters.

(c) *Procedure.* Proceed as directed in the United States Pharmacopeia XXI dissolution test. Ensure that all air is displaced from the immersed basket and that the contents of the pellet-filled capsule remain in the basket. Rotate the basket at the speed of 50 revolutions per minute for an accurately timed period of 30 minutes. Withdraw a 5-milliliter sample of the dissolution medium from a point midway between the stirring shaft and the wall of the

vessel and approximately midway in depth (this is the sample solution). Assay the sample solution for doxycycline as described in paragraph (d) of this section. Repeat the test on five additional pellet-filled capsules.

(d) *Doxycycline content*—(1) *Preparation of working standard solution.* Dissolve an accurately weighed portion of doxycycline hyclate working standard with 0.1*M* hydrochloric acid to obtain a concentration of 0.01 milligram per milliliter.

(2) *Preparation of sample solution.* Dilute the 5-milliliter sample portion to 25 milliliters with 0.1*M* hydrochloric acid.

(3) *Procedure.* Using a suitable spectrophotometer and 0.1*M* hydrochloric acid as the blank, determine the absorbance of each standard and sample solution at the absorbance peak at approximately 345 nanometers. Determine the exact position of the absorption peak for the particular instrument used.

(4) *Calculations.* Determine the total amount of doxycycline dissolved as follows:

$$\text{Milligrams of doxycycline dissolved} = \frac{A_u \times c \times d \times 900^*}{A_s}$$

where:

A_u=Absorbance of sample;

A_s=Absorbance of standard;

c=Concentration of working standard in milligrams; and

d=Dilution factor of sample withdrawn from beaker.

*If more than 15 milliliters of dissolution medium is removed, correct for the volume removed.

(e) *Evaluation.* Use the dissolution acceptance table and interpretation in the United States Pharmacopeia XXI.

[50 FR 41679, Oct. 15, 1985]

§ 436.545 Acid resistance test for erythromycin particles in tablets.

(a) *Equipment.* Use Apparatus 2 as described in the United States Pharmacopeia XXI dissolution test.

(b) *Acid resistance medium.* Use 0.1*N* hydrochloric acid, 500 milliliters.

(c) *Procedure.* Warm the immersion fluid to a temperature of 37±0.5 °C. Place one tablet into a vessel containing 500 milliliters of acid resistance medium. Rotate the paddle at the speed of 50 revolutions per minute for an accurately timed period of 1 hour. Withdraw a 50-milliliter sample of the dissolution medium from a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Filter the sample through a Whatman No. 1 filter paper or equivalent, discarding the first 5.0 milliliters. Assay for dissolved erythromycin as directed in paragraph (d) of this section using the filtrate as the sample solution. Repeat the test on five additional tablets.

(d) *Arsenomolybdate colorimetric assay for dissolved erythromycin*—(1) *Apparatus.* Automatic analyzer consisting of (i) a liquid sampler, (ii) a proportioning pump, (iii) suitable spectrophotometers equipped with matched flow cells and analysis capability at 660 nanometers, (iv) a means of recording spectrophotometric readings, and (v) a manifold consisting of the components illustrated in the diagram in paragraph (d)(4) of this section.

(2) *Reagents*—(i) *Arsenomolybdate solutions*—(*a*) *Stock solution.* Dissolve 100 grams of ammonium molybdate in approximately 1,700 milliliters of water contained in a 2-liter volumetric flask. Insert an inert plastic coated stirring bar into the flask, and begin mixing. While mixing, slowly add 84 milliliters of sulfuric acid (temperature of solution should not exceed 50 °C). Dissolve 12 grams of sodium arsenate in 100 milliliters of water, and add to the solution in the flask. Remove the stirring bar, dilute with water to volume, and mix. Store in an amber bottle for 24 hours before using. (This solution should not be allowed to come into contact with rubber.)

(*b*) *Working solution.* Dilute 1 part of stock solution with 2 parts of water, and mix. This solution is freshly prepared on the day of use.

(ii) *Acetate buffer, pH 4.8.* Dissolve 133 grams of ACS grade sodium acetate crystals in about 3.5 liters of water. Adjust the pH to 4.8±0.1 with glacial acetic acid. Dilute with water to 4,000 milliliters, and mix.

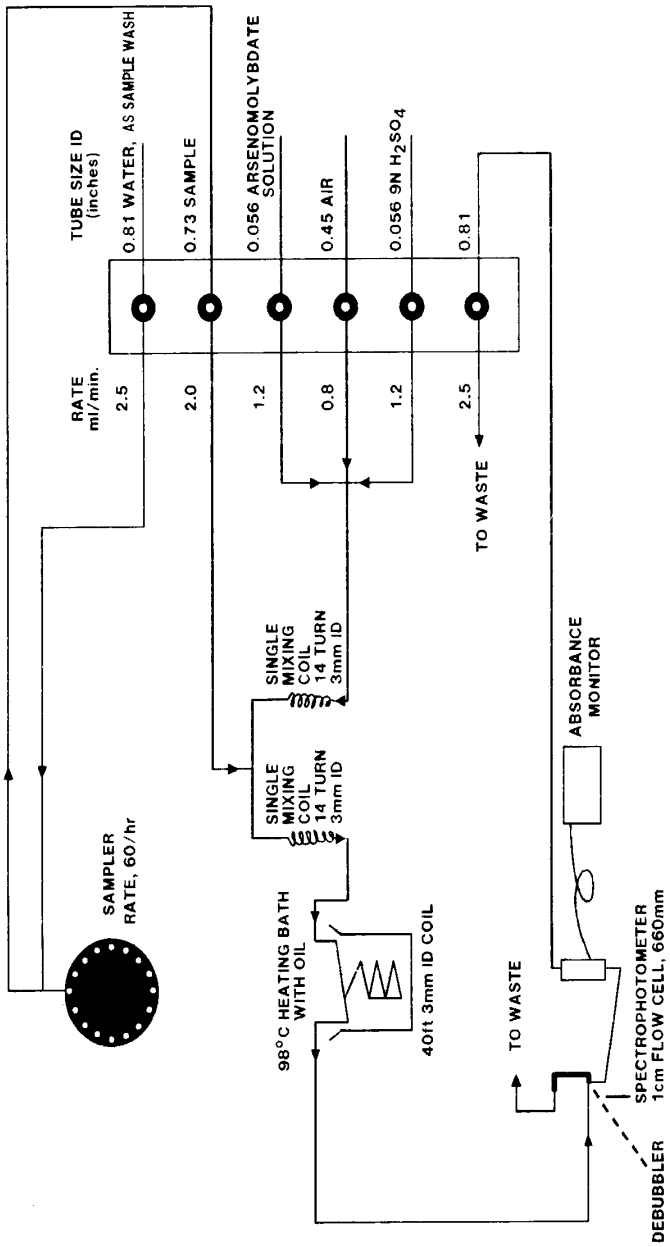
(iii) *9N Sulfuric acid.* Place a 2-liter volumetric flask containing an inert plastic coated magnetic stirring bar and about 1,500 milliliters of water in

an ice bath, and begin mixing. While mixing, cautiously add 300 milliliters of sulfuric acid. Allow the solution to cool. Remove the stirring bar, dilute with water to volume, and mix.

(3) *Preparation of working standard solutions*—(i) *Working standard stock solution.* Accurately weigh approximately 400 milligrams of USP Erythromycin Reference Standard, previously dried at 60 °C for 3 hours under vacuum (pressure of 5 millimeters of mercury or less), and transfer to a 100-milliliter volumetric flask. Dissolve and dilute with acetate buffer, pH 4.8 to volume, and mix.

(ii) *Working standard solutions.* Pipet 5, 10, 15, and 20 milliliters of the standard stock solution into separate 500-milliliter volumetric flasks, add acetate buffer, pH 4.8 to volume, and mix. The approximate concentrations of these solutions (before adjusting for the standard potency) are 40, 80, 120, and 160 micrograms of erythromycin per milliliter, respectively.

(4) *Procedure.* Use the working standard solutions prepared as described in paragraph (d)(3) of this section. The arrangement of the apparatus and flow of the samples and reagents are shown in the manifold diagram set forth following this paragraph. The sampler rate is usually 60 per hour, but may be varied. Establish a steady state by pumping reagents until the record trace becomes constant. Place cups containing the four concentrations of working standard solutions in the sampler followed by no more than 12 cups of sample solutions. Then place four more cups containing the four concentrations of working standard solutions in the sampler. Repeat the sequence above for additional samples by bracketing standards around no more than 12 sample solutions at a time.



(5) *System suitability test.* Perform a linear regression analysis of absorbance versus concentration in micrograms per milliliter of the standards. The system is suitable for calculation if the beginning baseline and the ending baseline after assaying a series of standard and sample solutions

does not vary by more than 2 percent transmittance, and the correlation coefficient for each standard curve is greater than 0.995.

(6) *Calculations.* (i) Calculate the concentration of each standard curve solution in micrograms of erythromycin per milliliter as follows:

$$\text{Concentration of each standard curve solution (micrograms of erythromycin per milliliter)} = \left(\frac{\text{Milligrams of working standard} \times \text{Potency of working standard (micrograms per milligram)}}{100}\right) \times \left(\frac{\text{Milliliters of standard stock solution}}{500}\right)$$

(ii) Calculate the percent of labeled amount of erythromycin released in 60 minutes as follows:

$$\text{Percent of labeled amount of erythromycin released in 60 minutes} = \left(\frac{500}{1{,}000}\right) \times \left(\frac{100}{\text{erythromycin content of tablet}}\right) \times \text{Micrograms of erythromycin per milliliter}$$

[51 FR 37721, Oct. 24, 1986]

PART 440—PENICILLIN ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
440.1a Sterile azlocillin sodium.
440.2a Sterile amdinocillin.
440.3 Amoxicillin trihydrate.
440.5 Ampicillin.
440.7 Ampicillin trihydrate.
440.7a Sterile ampicillin trihydrate.
440.8 Bacampicillin hydrochloride.
440.9a Sterile ampicillin sodium.
440.10 Benzylpenicilloyl-polylysine concentrate.
440.11 Carbenicillin indanyl sodium.
440.13a Sterile carbenicillin disodium.
440.15 Cloxacillin sodium monohydrate.
440.17 Cyclacillin.
440.19 Dicloxacillin sodium monohydrate.
440.19a Sterile dicloxacillin sodium monohydrate.
440.25 Hetacillin.
440.29 Hetacillin potassium.
440.29a Sterile hetacillin potassium.
440.36a Sterile methicillin sodium monohydrate.
440.37a Sterile mezlocillin sodium monohydrate.
440.41 Nafcillin sodium monohydrate.
440.41a Sterile nafcillin sodium monohydrate.
440.49 Oxacillin sodium monohydrate.
440.49a Sterile oxacillin sodium monohydrate.
440.55a Sterile penicillin G benzathine.
440.71 Penicillin V.
440.73 Penicillin V potassium.
440.74a Sterile penicillin G procaine.
440.80 Penicillin G potassium.
440.80a Sterile penicillin G potassium.
440.81a Sterile penicillin G sodium.
440.83a Sterile piperacillin sodium.
440.90a Sterile ticarcillin disodium.
440.91 Ticarcillin monosodium monohydrate.

Subpart B—Oral Dosage Forms

440.103 Amoxicillin oral dosage forms.
440.103a Amoxicillin trihydrate capsules.
440.103b Amoxicillin trihydrate for oral suspension.
440.103c Amoxicillin trihydrate chewable tablets.
440.103d Amoxicillin trihydrate and clavulanate potassium tablets.
440.103e Amoxicillin trihydrate and clavulanate potassium for oral suspension.
440.103f Amoxicillin trihydrate-clavulanate potassium chewable tablets.
440.105 Ampicillin oral dosage forms.
440.105a Ampicillin tablets.
440.105b Ampicillin chewable tablets.
440.105c Ampicillin capsules.
440.105d Ampicillin for oral suspension.
440.107 Ampicillin trihydrate oral dosage forms.
440.107a Ampicillin trihydrate chewable tablets.
440.107b Ampicillin trihydrate capsules.
440.107c Ampicillin trihydrate for oral suspension.
440.107d Ampicillin trihydrate-probenecid for oral suspension.

440.107e Ampicillin trihydrate-probenecid capsules.
440.108 Bacampicillin hydrochloride dosage forms.
440.108a Bacampicillin hydrochloride tablets.
440.108b Bacampicillin hydrochloride for oral suspension.
440.111 Carbencillin indanyl sodium tablets.
440.115 Cloxacillin sodium monohydrate oral dosage forms.
440.115a Cloxacillin sodium monohydrate capsules.
440.115b Cloxacillin sodium monohydrate for oral solution.
440.117 Cyclacillin oral dosage forms.
440.117a Cyclacillin tablets.
440.117b Cyclacillin for oral suspension.
440.119 Dicloxacillin sodium monohydrate oral dosage forms.
440.119a Dicloxacillin sodium monohydrate capsules.
440.119b Dicloxacillin sodium monohydrate for oral suspension.
440.125 Hetacillin oral dosage forms.
440.125a Hetacillin chewable tablets.
440.125b Hetacillin for oral suspension.
440.129 Hetacillin potassium capsules.
440.141 Nafcillin sodium monohydrate oral dosage forms.
440.141a Nafcillin sodium monohydrate tablets.
440.141b Nafcillin sodium monohydrate capsules.
440.141c Nafcillin sodium monohydrate for oral solution.
440.149 Oxacillin sodium monohydrate oral dosage forms.
440.149a Oxacillin sodium monohydrate capsules.
440.149b Oxacillin sodium monohydrate for oral solution.
440.155 Penicillin G benzathine oral dosage forms.
440.155a—440.155b [Reserved]
440.155c Penicillin G benzathine oral suspension.
440.155d Penicillin G benzathine tablets.
440.171 Penicillin V oral dosage forms.
440.171a Penicillin V capsules.
440.171b Penicillin V for oral suspension.
440.171c Penicillin V tablets.
440.173 Penicillin V potassium oral dosage forms.
440.173a Penicillin V potassium capsules.
440.173b Penicillin V potassium chewable tablets.
440.173c Penicillin V potassium tablets.
440.173d Penicillin V potassium for oral solution.
440.180 Penicillin G potassium oral dosage forms.
440.180a Penicillin G potassium tablets.
440.180c Penicillin G potassium capsules.
440.180f Penicillin G potassium for oral solution.
440.180g Penicillin G potassium tablets for solution.

Subpart C—Injectable Dosage Forms

440.201 Sterile azlocillin sodium.
440.202 Sterile amdinocillin.
440.207 Sterile ampicillin trihydrate for suspension.
440.209 Ampicillin sodium injectable dosage forms.
440.209a Sterile ampicillin sodium.
440.209b Sterile ampicillin sodium and sulbactam sodium.
440.210 Benzylpenicilloyl-polylysine injection.
440.213 Sterile carbenicillin disodium.
440.219 Dicloxacillin sodium monohydrate injectable dosage forms.
440.219a Sterile dicloxacillin sodium monohydrate.
440.219b Dicloxacillin sodium monohydrate for injection.
440.229 Hetacillin potassium injectable dosage forms.
440.229a Sterile hetacillin potassium.
440.229b Hetacillin potassium for injection.
440.236 Methicillin sodium monohydrate for injection.
440.237 Sterile mezlocillin sodium monohydrate.
440.241 Nafcillin sodium injectable dosage forms.
440.241a Nafcillin sodium monohydrate for injection.
440.241b Nafcillin sodium injection.
440.249 Oxacillin sodium injectable dosage forms.
440.249a Oxacillin sodium monohydrate for injection.
440.249b Oxacillin sodium injection.
440.255 Penicillin G benzathine injectable dosage forms.
440.255b Sterile penicillin G benzathine suspension.
440.255c Sterile penicillin G benzathine-penicillin G procaine suspension.
440.255d Sterile penicillin G benzathine for suspension.
440.274 Penicillin G procaine injectable dosage forms.
440.274a Sterile penicillin G procaine with aluminum stearate suspension.
440.274b Sterile penicillin G procaine suspension.
440.274c Sterile penicillin G procaine for suspension.
440.280 Penicillin G potassium injectable dosage forms.
440.280a Sterile penicillin G potassium.
440.280b Penicillin G potassium for injection.
440.280c Penicillin G potassium injection.
440.281 Penicillin G sodium injectable dosage forms.
440.281a Sterile penicillin G sodium.
440.281b Penicillin G sodium for injection.
440.283 Sterile piperacillin sodium.

440.290 Ticarcillin disodium injectable dosage forms.
440.290a Sterile ticarcillin disodium.
440.290b Sterile ticarcillin disodium and clavulanate potassium.
440.290c Ticarcillin disodium and clavulanate potassium injection.

Subparts D–J [Reserved]

Subpart K—Bulk Drug Formulations for Repacking or for Manufacturing Use

440.1080a Sterile penicillin G potassium buffered.
440.1081a Sterile penicillin G sodium, buffered.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§ 440.1a Sterile azlocillin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile azlocillin sodium is the sodium salt of 4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3,3-dimethyl-7-oxo-6-[[[[(2-oxo-1-imidazolidinyl)carbonyl] amino]phenylac-etyl]amino]-[2*S*-[2α,5α,6β(*S**)]]-. It is so purified and dried that:

(i) If the azlocillin sodium is not packaged for dispensing, its azlocillin content is not less than 859 micrograms and not more than 1,000 micrograms of azlocillin per milligram on an anhydrous basis. If the azlocillin sodium is packaged for dispensing, its azlocillin content is not less than 859 micrograms and not more than 1,000 micrograms of azlocillin per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 115 percent of the number of milligrams of azlocillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 2.5 percent.

(v) Its pH in an aqueous solution containing 100 milligrams of azlocillin per milliliter is not less than 6.0 and not more than 8.0.

(vi) Its specific rotation in an aqueous solution containing 10 milligrams of azlocillin per milliliter is +170° to +200°.

(vii) It gives a positive identity test for azlocillin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If it is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If it is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except:

(i) *Dilute Brij 35 solution*. In lieu of the hydroxylamine hydrochloride solution described in § 442.40(b)(1)(ii)(*b*)(*1*) of this chapter, use dilute Brij 35 solution in the reference channel. Prepare dilute Brij 35 solution as follows: Place 1 milliliter of Brij 35, 30 percent solution, into a 1-liter volumetric flask containing 900 milliliters of distilled water. Swirl gently and dilute to volume slowly with distilled water. Mix well.

(ii) *Buffer*. In lieu of the buffer described in § 442.40(b)(1)(ii)(*b*)(*2*) of this chapter, use the buffer prepared as follows: Dissolve 200 grams of primary standard tris (hydroxymethyl) aminomethane in sufficient distilled water to make 1 liter. Filter before use.

(iii) *Preparation of working standard solution*. Dissolve and dilute an accurately weighed portion of the azlocillin working standard with sufficient distilled water to obtain a concentration of 1.0 milligram of azlocillin per milliliter.

(iv) *Preparation of sample solutions*—(*a*) *Product not packaged for dispensing (micrograms of azlocillin per milligram).* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of 1.0 milligram of azlocillin per milliliter (estimated).

(*b*) *Product packaged for dispensing.* Determine both micrograms of azlocillin per milligram of the sample and milligrams of azlocillin per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(iv)(*b*)(*1*) and (*2*) of this section.

(*1*) *Micrograms of azlocillin per milligram.* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of 1.0 milligram of azlocillin per milliliter (estimated).

(*2*) *Milligrams of azlocillin per container.* Reconstitute as directed in the labeling using distilled water in lieu of the reconstituting fluid. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to a concentration of 1.0 milligram of azlocillin per milliliter (estimated).

(v) *Calculations*—(*a*) Calculate the micrograms of azlocillin per milligram of sample as follows:

$$\text{Micrograms of azlocillin per milligram of sample} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100_{-m})}$$

where:

A_u=Absorbance of sample solution;

P_s=Potency of working standard solution in micrograms per milliliter;

A_s=Absorbance of working standard solution;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture in sample.

(*b*) Calculate the azlocillin content of the single-dose vial as follows:

$$\text{Milligrams of azlocillin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;

P_s=Potency of working standard solution in micrograms per milliliter;

A_s=Absorbance of working standard solution; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of azlocillin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure and calculations described in paragraph (e)(2) of that section and preparing the sample as follows: Weigh the vial. Rapidly transfer a portion of the powder into the titration vessel, add the Karl Fischer reagent and restopper the vial immediately. Reweigh the vial to obtain the sample weight. A nitrogen purged glove bag or glove box should be used for preparing the sample.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of azlocillin per milliliter.

(6) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using an aqueous solution containing 10 milligrams of azlocillin per milliliter and a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(7) *Identity.* Proceed as directed in § 436.336 of this chapter.

[47 FR 53348, Nov. 26, 1982, as amended at 50 FR 1504, Jan. 11, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.2a Sterile amdinocillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile amdinocillin is 4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[(hexahydro-1*H*-azepin-1-yl)-methylene]amino]-3,3-dimethyl-7-oxo-, [2*S*-2α,5α,6β)]-. It is so purified and dried that:

(i) If the amdinocillin is not packaged for dispensing, its amdinocillin potency is not less than 950 micrograms and not more than 1,050 micrograms of amdinocillin per milligram on an anhydrous basis. If the amdinocillin is packaged for dispensing, its amdinocillin potency is not less than 950 micrograms and not more than 1,050 micrograms of amdinocillin per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of amdinocillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 0.5 percent.

(v) Its pH in an aqueous solution containing 100 milligrams of amdinocillin per milliliter is not less than 4.0 and not more than 6.2.

(vi) It is crystalline.

(vii) It gives a positive identity test for amdinocillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for amdinocillin potency, and if packaged for dispensing, amdinocillin potency and container content, sterility, pyrogens, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If it is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If it is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 25 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Amdinocillin potency and container content.* Proceed as directed in § 436.353 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 220 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, e.g., a Whatman ODS–3 column (25-centimeter column having an inside diameter of 4.6 millimeters and 5 micrometer particle size or equivalent), a flow rate of 1.0 milliliter per minute, and an injection volume of 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Buffer solution 0.01M pH 5.0.* Transfer 1.36 grams of monobasic potassium phosphate in sufficient water to make 1,000 milliliters of solution. Adjust the pH to 5.0 ± 0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide.

(*b*) *Mobile phase.* Mix acetonitrile (high-pressure liquid chromatography grade): 0.01*M* pH 5.0 phosphate buffer (15:85).

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Prepare the working standard solution fresh before injection by dissolving an accurately weighed portion of the amdinocillin working standard with sufficient distilled water to obtain a stock solution containing approximately 100 micrograms of amdinocillin per milliliter.

(*b*) *Preparation of sample solutions*—(*1*) *Product not packaged for dispensing (micrograms of amdinocillin per milligram).* Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(*2*) *Product packaged for dispensing.* Determine both micrograms of amdinocillin per milligram of the sample and milligrams of amdinocillin per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(ii)(*b*)(*2*)(*i*) and (*ii*) of this section.

(*i*) *Micrograms of amdinocillin per milligram.* Dissolve an accurately weighed

portion of the sample with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(*ii*) *Milligrams of amdinocillin per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 100 micrograms of amdinocillin per milliliter (estimated).

(*iii*) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2.5 at 5 percent of peak height:

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the peak for amdinocillin and its nearest eluting impurity is satisfactory if it is not less than 2.5.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in § 436.353(b) of this chapter.

(iv) *Calculations.* (*a*) Calculate the micrograms of amdinocillin per milligram of sample as follows:

$$\text{Micrograms of amdinocillin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - \text{m})}$$

where:

A_u=Area of the amdinocillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the amdinocillin peak in the chromatogram of the amdinocillin working standard;

P_s=Amdinocillin activity in the amdinocillin working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(*b*) Calculate the amdinocillin content of the container as follows:

$$\text{Milligrams of amdinocillin per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the amdinocillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the amdinocillin peak in the chromatogram of the amdinocillin working standard:

P_s=Amdinocillin activity in the amdinocillin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 40 milligrams of amdinocillin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of amdinocillin per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a potassium bromide disc containing 1 milligram of amdinocillin in 300 milligrams of potassium bromide, prepared as described in paragraph (b)(l) of that section.

[50 FR 7765, Feb. 26, 1985; 50 FR 10220, Mar. 14, 1985; 50 FR 18243, Apr. 30, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.3 Amoxicillin trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate is the trihydrate form of D(−) $_a$-amino-*p*-hydroxybenzyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of amoxicillin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 11.5 percent and not more than 14.5 percent.

(iv) Its pH in an aqueous solution containing 2 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its amoxicillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent amoxicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than 6. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent amoxicillin content of the sample determined by the nonaqueous acid titration is not more than 6. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent amoxicillin content of the sample determined by the nonaqueous base titration is not more than 6.

(vii) It is crystalline.

(viii) It gives a positive identity test for amoxicillin trihydrate.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement: ''For use in the manufacture of nonparenteral drugs only''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the iodometric assay shall be conclusive:

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 1.0 milligram of amoxicillin per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of amoxicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 2 milligrams per milliliter.

(5) *Amoxicillin content.* Proceed as directed in § 436.213 of this chapter using both the titration procedures described in paragraphs (e) (1) and (2) of that section. Calculate the percent amoxicillin content as follows:

(i) *Acid titration.*

$$\text{Percent amoxicillin content} = \frac{(A - B)(\text{normality of lithium methoxide reagent})\,(365.4)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A=Milliliters of lithium methoxide reagent used in titrating the sample.

B=Milliliters of lithium methoxide reagent used in titrating the blank.

m=Percent moisture content of the sample.

Calculate the difference between the potency and the amoxicillin content as follows:

(ii) *Base titration.*

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent amoxicillin content}$$

$$\text{Percent amoxicillin content} = \frac{(A - B)(\text{normality of perchloric acid reagent})(365.4)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample.

B=Milliliters of perchloric acid reagent used in titrating the blank.

m=Percent moisture content of the sample.

Calculate the difference between the potency and the amoxicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent amoxicillin content}$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[39 FR 34032, Sept. 23, 1974, as amended at 46 FR 16682, Mar. 13, 1981; 49 FR 3458, Jan. 27, 1984; 50 FR 19918, May 13, 1985]

§ 440.5 Ampicillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin is 6-[D-$_a$-aminobenzyl] penicillin. It is a white powder. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of ampicillin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than six. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than six. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than six.

(vii) It is crystalline.

(viii) It gives a positive identity test for ampicillin.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement, ''For use in the manufacture of nonparenteral drugs only''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately

weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(a) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Ampicillin content*. Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraphs (e) (1) and (2) of that section. Calculate the percent ampicillin content as follows:

(i) *Acid titration*.

$$\text{Percent ampicillin content} = \frac{(\text{A} - \text{B})(\text{normality of lithium methoxide reagent})(349.4)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A = Milliliters of lithium methoxide reagent used in titrating the sample;

B = Milliliters of lithium methoxide reagent used in titrating the blank;

m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}$$

(ii) *Base titration*.

$$\text{Percent ampicillin content} = \frac{(\text{A} - \text{B})(\text{normality of perchloric acid reagent})(349.4)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A = Milliliters of perchloric acid reagent used in titrating the samples;

B = Milliliters of perchloric acid reagent used in titrating the blank;

m = Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}$$

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 41 FR 42649, Sept. 28, 1976; 46 FR 16682, Mar. 13, 1981; 49 FR 3458, Jan. 27, 1984; 50 FR 19918, May 13, 1985]

§ 440.7 Ampicillin trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin trihydrate is the trihydrate form of D(−)$_a$-amino-benzyl penicillin. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050

micrograms of ampicillin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not less than 12 percent and not more than 15 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than 6. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than 6. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6.

(vii) It is crystalline.

(viii) It gives a positive identity test for ampicillin trihydrate.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, this drug shall be labeled ''ampicillin'' and each package shall bear on its outside wrapper or container and the immediate container the following statement ''For use in the manufacture of nonparenteral drugs only''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required: 10 packages each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraphs (e) (1) and (2) of that section. Calculate the percent ampicillin content as follows:

(i) *Acid titration.*

Percent ampicillin content=[(A−B) (normality of lithium methoxide reagent) (349.4) (100) (100)]/[(Weight of sample in milligrams) (100−*m*)],

where:

A=Milliliters of lithium methoxide reagent used in titrating the sample;

B=Milliliters of lithium methoxide reagent used in titrating the blank;

m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

Difference=(Potency in micrograms per milligram/10)−percent ampicillin content.

(ii) *Base titration.*

Percent ampicillin content=[(A−B) (normality of perchloric acid reagent) (349.4) (100) (100)]/[(Weight of sample in milligrams) (100−*m*)],

where:

A=Milliliters of perchloric acid reagent used in titrating the samples;
B=Milliliters of perchloric acid reagent used in titrating the blank;
m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

Difference=(Potency in micrograms per milligram/10)−percent ampicillin content.

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using an 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 46 FR 16683, Mar. 13, 1981; 49 FR 3458, Jan. 27, 1984; 50 FR 19918, May 13, 1985]

§ 440.7a Sterile ampicillin trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin trihydrate is the trihydrate form of D(-)α-aminobenzyl penicillin. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of ampicillin per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not less than 12 percent and not more than 15 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(vii) Its ampicillin content is not less than 90 percent on an anhydrous basis.

(viii) The acid-base titration concordance is such that the difference between the percent ampicillin content when determined by nonaqueous acid titration and by nonaqueous base titration is not more than 6. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous acid titration is not more than 6. The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6.

(ix) It is crystalline.

(x) It gives a positive identity test for ampicillin trihydrate.

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, this drug shall be labeled ''ampicillin.''

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive:

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter. Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3) to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except in lieu of paragraph (e)(1)(i)(*a*), prepare the sample for test as follows: From each of 10 immediate

containers, aseptically transfer approximately 300 milligrams of sample into a sterile 500-milliliter Erlenmeyer flask containing approximately 400 milliliters of diluting fluid D. Add at least 200,000 Levy units[1] of penicillinase. Repeat the process using 10 additional containers. Swirl both of the stoppered flasks to completely solubilize the suspension prior to filtration and proceed as directed in paragraph (e)(1)(ii) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(f) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraph (e)(1) and (2) of that section. Calculate the ampicillin content as follows:

(i) *Acid titration.*

Percent ampicillin content=[(A − B) (normality of lithium methoxide reagent) (349.4) (100) (100)]/[(Weight of sample in milligrams) (100 − *m*)].

where:

A=Milliliters of lithium methoxide reagent used in titrating the sample;

B=Milliliters of lithium methoxide reagent used in titrating the blank;

m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

Difference=(Potency in micrograms per milligram/10) − percent ampicillin content.

(ii) *Base titration.*

Percent ampicillin content=[(A − B) (normality of perchloric acid reagent) (349.4) (100) (100)]/[(Weight of sample in milligrams) (100 − *m*)].

where:

A=Milliliters of perchloric acid reagent used in titrating the samples;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

Difference=(Potency in micrograms per milligram/10) − percent ampicillin content.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc, prepared as described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 46 FR 16683, Mar. 13, 1981; 49 FR 3458, Jan. 27, 1984; 50 FR 19918, May 13, 1985]

§ 440.8 Bacampicillin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacampicillin hydrochloride is the hydrochloride salt of the 1–ethoxycarbonyloxyethyl ester of ampicillin. It is a white powder. It is so purified and dried that:

(i) Its potency is not less than 623 micrograms and not more than 727 micrograms of ampicillin per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 20 milligrams per milliliter is not less than 3.0 and not more than 4.5.

(v) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except:

[1] One Levy unit of penicillinase inactivates 59.3 units of penicillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

(*a*) *Buffer.* In lieu of the buffer described in § 442.40(b)(1)(ii) (*b*)(*2*) of this chapter, use the buffer prepared as follows: Dissolve 200 grams of primary standard tris (hydroxymethyl) aminomethane in sufficient distilled water to make 1 liter. Filter before use.

(*b*) *Preparation of working standard solution.* Use the ampicillin working standard. Dissolve and dilute an accurately weighed portion of the ampicillin working standard in sufficient distilled water to obtain a concentration of 1.25 milligrams of ampicillin per milliliter.

(*c*) *Preparation of sample solution.* Dissolve and dilute an accurately weighed portion of the sample with sufficient distilled water to obtain a concentration of 1.25 milligrams of ampicillin per milliliter (estimated).

(*d*) *Calculations.* Calculate the ampicillin content in micrograms per milligram as follows:

$$\text{Ampicillin content in micrograms per milligram} = \frac{A_u \times P_a}{A_s \times W_u}$$

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except use the ampicillin working standard.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 20 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.330 of this chapter.

[46 FR 25603, May 8, 1981, as amended at 50 FR 19918, May 13, 1985]

§ 440.9a Sterile ampicillin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile ampicillin sodium is the sodium salt of D(-)$_a$-aminobenzyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 845 micrograms and not more than 988 micrograms of ampicillin per milligram on an anhydrous basis. If it is packaged for dispensing, it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 8.0 and not more than 10.0.

(vii) Its ampicillin content is not less than 84.5 percent, except if the high-performance liquid chromatographic (HPLC) assay method is used, then the ampicillin content standard is not applicable.

(viii) The potency-base titration concordance is such that the difference between the potency value divided by 10 and the percent ampicillin content of the sample determined by the nonaqueous base titration is not more than 6, except if the HPLC assay method is used, then the concordance standard is not applicable.

(ix) It is crystalline.

(x) It passes the identity test for ampicillin sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, ampicillin content, concordance, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in manufacturing another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers or if each vial contains 250 milligrams or less of ampicillin a minimum of 24 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution containing 0.1 milligram of ampicillin per milliliter (estimated), for the microbiological agar diffusion assay and in 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the iodometric assay or for the hydroxylamine colorimetric assay to give a stock solution of convenient concentration. For the high-performance liquid chromatographic assay (HPLC), transfer an accurately weighed portion of ampicillin, equivalent to about 100 milligrams of anhydrous ampicillin, to a 100-milliliter volumetric flask. Add about 75 milliliters of diluent (prepared as described in paragraph (b)(1)(ii)(*d*)(*1*)(*ii*) of this section), shake and sonicate, if necessary, to achieve complete dissolution. Also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either sterile distilled water, solution 1, or HPLC diluent to give a stock solution as specified above.

(ii) *Assay procedure.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution.

(*c*) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(*d*) *HPLC assay*. Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4-millimeter X 5-centimeter guard column containing 40- to 60-micrometer diameter packing material as described for the analytical column, a 4-millimeter X 30-centimeter analytical column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, and a flow rate of about 2.0 milliliters per minute. Separately inject equal volumes (about 20 microliters) of the working standard preparation and the sample solution into the chromatograph, record the chromatogram, and measure the responses for the major peaks. Reagents, working standard and resolution test solution, system suitability requirements, and calculations are as follows:

(*1*) *Reagents*—(*i*) *Mobile phase.* Prepare a suitably filtered and degassed mixture of water, acetonitrile, 1.0*M* monobasic potassium phosphate, and 1.0*N* acetic acid (909:80:10:1).

(*ii*) *Diluent.* Mix 10 milliliters of 1.0*M* monobasic potassium and 1 milliliter of 1.0*N* acetic acid, dilute with water to make 1,000 milliliters, and mix.

(*2*) *Preparation of working and internal standard solutions*—(*i*) *Working standard solution*. Dissolve a portion of ampicillin working standard, accurately weighed, in the diluent to obtain a solution having a known concentration of about 1 milligram per milliliter. Shake and sonicate, if necessary, to achieve complete dissolution. Use this solution promptly after preparation.

(*ii*) *Resolution test solution*. Dissolve caffeine in working standard solution to obtain a solution containing about 1 milligram per milliliter.

(*3*) *System suitability requirements*—(*i*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.4 at 5 percent of peak height.

(*ii*) *Resolution.* The resolution (*R*) between the caffeine and the ampicillin peaks is satisfactory if it is not less

than 2.0. The relative retention times are about 2.0 for caffeine and 1.0 for ampicillin.

(*iii*) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(i) of this section should not be changed.

(*4*) *Calculations.* Calculate the micrograms of ampicillin per milligram of sample as follows:

$$\text{Micrograms of ampicillin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the ampicillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ampicillin peak in the chromatogram of the ampicillin working standard;

P_s=Ampicillin activity in the ampicillin working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ampicillin per milliliter.

(7) *Ampicillin content.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the ampicillin content as follow

$$\text{Percent ampicillin content} = \frac{(\text{A} - \text{B})(\text{normality of perchloric acid reagent})(174.7)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture content of the sample.

Calculate the difference between the potency and the ampicillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent ampicillin content}$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

[39 FR 18976, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 41 FR 42649, Sept. 28, 1976; 42 FR 59857, Nov. 22, 1977; 46 FR 16683, Mar. 13, 1981; 49 FR 3458, Jan. 27, 1984; 50 FR 19918, May 13, 1985; 52 FR 42288, Nov. 4, 1987; 52 FR 45281, Nov. 25, 1987; 54 FR 47204, Nov. 13, 1989]

§ 440.10 Benzylpenicilloyl-polylysine concentrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Benzylpenicilloyl-polylysine concentrate is a pale yellow to dark yellow aqueous solution of benzylpenicilloyl *e* substituted poly-L-lysine. It contains one or more suitable and harmless buffers. It is so purified that:

(i) It contains not less than 50 percent and not more than 70 percent benzylpenicilloyl substitution on the polylysine.

(ii) The benzylpenicilloyl concentration is not less than $1.25\times10^{-2}M$ and not more than $2.0\times10^{-2}M$.

(iii) The penamaldate concentration is not more than $6.0\times10^{-4}M$.

(iv) The penicillenate concentration is not more than $2.0\times10^{-4}M$.

(v) Its pH is not less than 6.5 and not more than 8.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for percent benzylpenicilloyl substitution, benzylpenicilloyl content, penamaldate content, penicillenate content, and pH.

(ii) Samples required: 2 vials, each containing not less than 5 milliliters.

(b) *Tests and methods of assay*—(1) *Percent benzylpenicilloyl substitution*—(i) *Lysine content*—(*a*) *Equipment.* Amino acid analyzer capable of:

(*1*) Separating the hydrolysis products of benzylpenicilloyl polylysine into discrete components by means of an ion-exchange column.

(*2*) Mixing the separated amino acid components with a ninhydrin reagent and promoting the reaction in a coil at elevated temperatures.

(*3*) Quantitating the ninhydrin positive materials by means of a suitable colorimeter and recorder.

(*b*) *Reagents*—(*1*) Citrate buffer: Dissolve and dilute 19.69 grams of sodium citrate dihydrate, 16.5 milliliters of hydrochloric acid, 0.1 milliliter of pentachlorophenol, 5 milliliters of thiodiglycol in 900 milliliters of distilled water; adjust to a pH of 2.2 and dilute to 1 liter with distilled water.

(*2*) Calibration mixture: Dissolve and dilute equal molar amounts of ammonia, and the L form of lysine in the citrate buffer to result in final concentrations of $2.5\times10^{-4}M$ for each.

(*c*) *Preparation of standard and sample solutions*—(*1*) *Standard solution* (*standard lysine solution* (2.5×10^{-4} *M*)). Transfer an accurately weighed portion of 54.8 milligrams of lysine dihydrochloride to a 100-milliliter volumetric flask. Dissolve and dilute to mark with citrate buffer. Make an accurate tenfold dilution of this solution with citrate buffer. The resulting standard solution is 2.5×10^{-4} *M* with respect to lysine.

(*2*) *Sample solution.* Dilute 1 milliliter of the benzylpenicilloyl-polylysine concentrate to 10 milliliters with distilled water. Mix 1 milliliter of the diluted solution with 1.5 milliliters of 6.0*N* hydrochloric acid and seal in an ampule under nitrogen. Hydrolyze the solution for 22 hours at 110° C. Transfer the contents of the ampule quantitatively into a 50-milliliter round bottom flask and dry by rotary evaporation. Wash the contents and evaporate to dryness three times using 5-milliliter portions of distilled water. Dissolve the hydrolysate in 10 milliliters of citrate buffer.

(*d*) *Procedure.* Standardize the procedure for use of the amino acid analyzer with the calibration mixture. Apply 0.5 milliliter of the lysine standard solution to the amino acid analyzer and determine the area of the lysine peak. Apply 0.5 milliliter of the sample solution to the amino acid analyzer and determine the area of the lysine peak.

(*e*) *Calculations.* Calculate the lysine content by the following formula:

$$\text{Molar concentration of lysine in the benzylpenicilloyl-polylysine concentrate} = \frac{A\times2.5}{B\times C}$$

where:

A=The area of the lysine peak of the sample solution.

B=The area of the lysine peak of the standard solution.

C=The percent purity of the lysine dihydrochloride.

(ii) *Benzylpenicilloyl content*—(*a*) *Reagents.* (*1*) Mercuric chloride solution: Dissolve 35 milligrams of mercuric

chloride in 500 milliliters of distilled water.

(*2*) Saline phosphate buffer, pH 7.6: Dissolve 9 grams of sodium chloride and 1.38 grams monobasic sodium phosphate in 900 milliliters of distilled water, adjust to pH 7.6 and dilute to 1 liter with distilled water.

(*b*) *Preparation of sample solution.* Transfer 1 milliliter of the benzylpenicilloyl-polylysine concentrate into a 500-milliliter volumetric flask and dilute to volume with saline phosphate buffer, pH 7.6.

(*c*) *Procedure.* Transfer 3 milliliters of the sample solution into a spectrophotometric cell. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the initial absorbance at 282 nanometers. Thereafter, react the diluted benzylpenicilloyl-polylysine solution with 0.02-milliliter portions of the mercuric chloride solution. Determine the absorbance at 282 nanometers at 1 and 3 minutes after each addition of mercuric chloride solution. The increased absorbance at 282 nanometers is used in calculating the benzylpenicilloyl content. Calculate the benzylpenicilloyl content by means of the following formula:

$$\text{Molar benzyl-penicilloyl content} = \frac{(A_1 - A_2) \times 500}{22{,}325}$$

where:

A_1=The highest absorbance at 282 nanometers

A_2=The initial absorbance at 282 nanometers

22,325=The molar absorptivity of the penamaldate formed by the reaction of the benzylpenicilloyl moiety with the mercuric chloride at a pH of 7.6.

Percent benzylpenicilloyl substitution=(Molar benzylpenicilloyl content ×100)/Molar lysine content

(2) *Penicillenate and penamaldate content.* Dilute 1 milliliter of the benzylpenicilloyl-polylysine concentrate to 50 milliliters with saline phosphate buffer, pH 7.6. Using a suitable spectrophotometer and the saline phosphate buffer, pH 7.6, as a blank, determine the absorbance at 322 and 282 nanometers. Calculate the penicillenate content by the following formula:

$$\text{Molar penicillenate content} = \frac{\text{Absorbance at 322 nanometers} \times 50}{26{,}600}$$

where:

26,600=Molar absorptivity of the penicillenate moiety at 322 nanometers at a pH of 7.6

Calculate the penamaldate content by the following formula:

$$\text{Molar penamaldate content} = \frac{\text{Absorbance at 282 nanometers} \times 50}{22{,}325}$$

where:

22,325=Molar absorptivity of the penamaldate moiety at 282 nanometers at a pH of 7.6.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 35346, Oct. 1, 1974; 39 FR 38370, Oct. 31, 1974; 39 FR 39871, Nov. 12, 1974; 39 FR 40946, Nov. 22, 1974]

§ 440.11 Carbenicillin indanyl sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Carbenicillin indanyl sodium is the monosodium salt of *N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] hept-6-yl)-2-phenylmalonamic acid, 1-(5-indanyl) ester. It is so purified and dried that:

(i) Its potency is not less than 659 micrograms and not more than 769 micrograms of carbenicillin per milligram on an anhydrous basis at the time of certification, and not less than 630 micrograms of carbenicillin per milligram on an anhydrous basis at any time during the expiration period.

(ii) [Reserved]

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 5.0 nor more than 8.0.

(v) It gives a positive result to the identity test for carbenicillin indanyl sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples required: Five packages, each containing approximately 1.0 gram and one package containing approximately 2.5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.300 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 50 FR 19918, May 13, 1985]

§ 440.13a Sterile carbenicillin disodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Carbenicillin disodium is the disodium salt of α-carboxybenzylpenicillin. It is so purified and dried that:

(i) It contains not less than 770 micrograms of carbenicillin per milligram on an anhydrous basis. If it is packaged for dispensing, its carbenicillin content is not less than 90 percent and not more than 120 percent of the number of milligrams of carbenicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of carbenicillin per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 6.0 and not more than 8.0.

(vii) It gives a positive identity test for carbenicillin disodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single–dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. If it is a single dose container, use a separate needle and syringe for each container. Dilute with sufficient solution 1 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 20.0 micrograms of carbenicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 200 milligrams of carbenicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in §436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams of carbenicillin per milliliter (or if packaged for dispensing, use a solution prepared as directed for reconstitution in the labeling).

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 45 FR 22921, Apr. 4, 1980; 50 FR 19918, May 13, 1985; 51 FR 27532, Aug. 1, 1986]

§440.15 Cloxacillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cloxacillin sodium is the monohydrate sodium salt of 5-methyl-3-(*o*- chlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 825 micrograms of cloxacillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3 percent and not more than 5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.5 nor more than 7.5.

(v) Its cloxacillin content is not less than 82.5 percent.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, cloxacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in §436.204 of this subchapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in §436.205 of this subchapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this subchapter.

(4) *pH.* Proceed as directed in §436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Cloxacillin content.* Accurately weigh approximately 100 milligrams of the sample and dissolve in sufficient 5*N* sodium hydroxide to give a total volume of 25 milliliters. Place in a boiling water bath for 30 minutes. Cool, acidify 1 milliliter with 1 milliliter of dilute sulfuric acid (1 in 2), add 8 milliliters of water, and extract with two 25-milliliter portions of ethyl ether. Combine the ether extractives and extract with 25-milliliter portions of 0.1*N* sodium hydroxide. Combine the alkaline extractives and dilute to 100 milliliters with carbon dioxide-free water. Treat a portion of the cloxacillin working standard in the same manner. Using a suitable spectrophotometer, determine the absorbance of the solution in a 1-centimeter cell at the absorption peaks at 257±3 nanometers and at 282±3 nanometers compared with a reagent blank. Determine the percent

cloxacillin in the sample by means of the following calculation:

$$\text{Percent cloxacillin} = \frac{A_1 \times \text{weight of standard in milligrams, on an "as is" basis} \times \text{percent cloxacillin in the standard}}{A_2 \times \text{weight of sample in milligrams on an "as is" basis} \times 100}$$

where:

A_1=Difference in absorbance for the sample between 257 nanometers and 282 nanometers:

A_2=Difference in absorbance for the cloxacillin working standard, similarly treated.

(6) *Identity.* Proceed as directed in § 436.211 of this subchapter, using the 0.5 percent potassium bromide disc described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203 of this subchapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 50 FR 19918, May 13, 1985]

§ 440.17 Cyclacillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclacillin is 6-(1-aminocyclohexanecarboxamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. It is a white to off-white powder. It is so purified and dried that:

(i) It contains not less than 900 micrograms and not more than 1,050 micrograms of cyclacillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 6.5.

(v) Its cyclacillin content is not less than 90 percent on an anhydrous basis.

(vi) The acid-base titration concordance is such that the difference between the percent cyclacillin content when determined by nonaqueous acid titration and nonaqueous base titration is not more than six. The potency-acid titration concordance is such that the difference between the potency value divided by 10 and the percent cyclacillin content of the sample determined by the nonaqueous acid titration is not more than six. The potency base titration concordance is such that the difference between the potency value divided by 10 and the percent cyclacillin content of the sample determined by the nonaqueous base titration is not more than six.

(vii) It is crystalline.

(viii) It gives a positive identity test for cyclacillin.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the following statement, ''For use in the manufacture of nonparenteral drugs only.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, cyclacillin content, concordance, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution containing 1 milligram of cyclacillin per milliliter (estimated). Further dilute an aliquot of the stock

solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of cyclacillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Cyclacillin content*. Proceed as directed in § 436.213 of this chapter, using both the titration procedures described in paragraph (e)(1) and (2) of that section. Calculate the percent cyclacillin content as follows:

(i) *Acid titration*.

$$\text{Percent cyclacillin content} = \frac{(A - B)(\text{normality of perchloric acid reagent})(341.4)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A = Milliliters of lithium methoxide reagent used in titrating the sample;

B = Milliliters of lithium methoxide reagent used in titrating the blank;

m = Percent moisture content of the sample.

Calculate the difference between the potency and the cyclacillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligram}}{10} - \text{percent cyclacillin content}$$

(ii) *Base titration*.

$$\text{Percent cyclacillin content} = \frac{(A - B)(\text{normality of perchloric acid reagent})\,(341.4)(100)(100)}{(\text{Weight of sample in milligrams})(100 - m)}$$

where:

A = Milliliters of perchloric acid reagent used in titrating the sample;

B = Milliliters of perchloric acid reagent used in titrating the blank;

m = Percent moisture content of the sample.

Calculate the difference between the potency and the cyclacillin content as follows:

$$\text{Difference} = \frac{\text{Potency in micrograms per milligrams}}{10} - \text{percent cyclacillin content}$$

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 1-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[46 FR 2981, Jan. 13, 1981; 46 FR 15880, Mar. 10, 1981, as amended at 50 FR 19918, May 13, 1985]

§ 440.19 Dicloxacillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Dicloxacillin sodium monohydrate is the monohydrated sodium salt of 5-methyl-3-(2,6-dichlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of dicloxacillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3 percent nor more than 5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.5 nor more than 7.5.

(v) Its organic chlorine content is not less than 13.0 percent nor more than 14.2 percent.

(vi) Its free chloride content is not more than 0.5 percent.

(vii) It is crystalline.

(viii) It gives a positive identity test for dicloxacillin sodium monohydrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(ii) Samples required: 10 containers, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay, as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture content.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Organic chlorine content*—(i) *Reagents.* (*a*) *o*- Chlorobenzoic acid of known purity.

(*b*) 0.01*N* Silver nitrate solution. Store in brown glass reagent bottle. Standardize against an accurately weighed sample of 20 to 25 milligrams of *o*- chlorobenzoic acid using the procedure described in paragraph (b)(5)(ii) of this section.

$$\text{Normality } (N) = \frac{\text{Percent purity of the } o\text{-chlorobenzoic acid} \times \text{milligrams of } o\text{-chlorobenzoic acid}}{15{,}657 \times \text{milliliters of silver nitrate consumed}}$$

(*c*) 0.1*N* Sodium hydroxide solution.

(*d*) 1:1 Nitric acid solution: Mix 1 volume of concentrated nitric acid with 1 volume of distilled water.

(ii) *Total chlorine.* (Caution—The analyst should wear safety glasses and use a suitable shield between himself and the apparatus. The glassware must be scrupulously clean.) Accurately weigh 20 to 25 milligrams of the sample and place it on the center of a piece of halide-free filter paper measuring about 4 centimeters square (this is specially cut paper with a fuse strip attached to the area that holds the sample), and fold the paper to enclose it. Place 10 milliliters of 0.1*N* sodium hydroxide into an oxygen combustion flask (Schoniger flask), and flush the air from the flask with a stream of rapidly flowing oxygen. Place the sample into the platinum sample holder and ignite the fuse strip by suitable means. If the strip is ignited outside the flask, immediately plunge the stopper into the flask, invert so that the sodium hydroxide solution makes a seal around

the stopper, and hold the stopper firmly in place. If the ignition is carried out in a closed system, the inversion of the flask may be omitted. After combustion is completed, shake the flask vigorously, add a small amount of distilled water to the collar to insure an air tight seal, and allow to stand for not less than 10 minutes with intermittent shaking. Transfer to a suitable titration vessel, heat on a steam bath for 20 to 30 minutes, cool to room temperature, add 5 milliliters of nitric acid solution, and titrate potentiometrically with 0.01*N* silver nitrate, using one silver electrode and one silver/silver chloride electrode.

$$\text{Percent total chlorine} = \frac{N \times \text{milliliters of silver nitrate} \times 3545.7}{\text{Milligrams of sample}}$$

(iii) *Free chloride.* Accurately weigh 100 to 150 milligrams of sample directly into a titration flask, dissolve in 10 milliliters of 0.1*N* sodium hydroxide, and add about 20 milliliters of distilled water, heat this solution on the steam bath 20 to 30 minutes. Cool to room temperature, add 5 milliliters of 1:1 nitric acid solution and titrate potentiometrically with 0.01*N* silver nitrate using one silver electrode and one silver/silver chloride electrode.

$$\text{Percent free chloride} = \frac{N \times \text{milliliters of silver nitrate} \times 3545.7}{\text{Milligrams of sample}}$$

(iv) *Organic chlorine.* Percent organic chlorine=Percent total chlorine−percent free chloride.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 1 percent potassium bromide disc described in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 44 FR 10378, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.19a Sterile dicloxacillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile dicloxacillin sodium monohydrate is the monohydrated sodium salt of 5-methyl-3-(2,6-dichlorophenyl)-4-isoxazolyl penicillin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of dicloxacillin per milligram. If it is packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of dicloxacillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 3 percent and not more than 5 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter or when reconstituted as directed in the labeling, if it is packaged for dispensing is not less than 4.5 nor more than 7.5.

(vii) Its organic chlorine content is not less than 13.0 percent and not more than 14.2 percent.

(viii) Its free chloride content is not more than 0.5 percent.

(ix) It is crystalline.

(x) It gives a positive identity test for dicloxacillin sodium monohydrate.

(2) *Labeling.* If this drug is packaged for dispensing, in addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''sterile dicloxacillin sodium''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay and the hydroxylamine colorimetric assay or in distilled water for the iodometric assay, to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water, as specified above, to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of dicloxacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter (or using a solution reconstituted as directed in the labeling if it is packaged for dispensing).

(7) *Organic chlorine content.* Proceed as directed in § 440.19(b)(5).

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 50 FR 19918, May 13, 1985]

§ 440.25 Hetacillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin is 6-(2,2-Dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It occurs as a fine, white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 810 micrograms of ampicillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.5 nor more than 5.5.

(v) Its hetacillin content is not less than 90 and not more than 105 percent.

(vi) It gives a positive identity test for hetacillin.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Hetacillin content*—(i) *Reagents*—(*a*) *Hydrochloric acid-acetone solution.* Dilute 8.5 milliliters of concentrated hydrochloric acid to 1 liter with acetone and mix well. Use for 1 day only.

(*b*) *p-Dimethylaminocinnamalde- hyde solution.* Dissolve 0.5 gram of *p*-dimethylaminocinnamaldehyde in sufficient hydrochloric acid-acetone solution to a final volume of 100 milliliters and shake well, filtering if necessary. Prepare immediately before use.

(ii) *Preparation of standard solutions.* Transfer about 100 milligrams of the hetacillin working standard, accurately weighed, to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 0.5, 1.0, and 2.0 milliliters into three respective 25-milliliter volumetric flasks. Add 1.5 and 1.0 milliliters of 0.1*N* hydrochloric acid respectively to the first and second flasks to bring the volume in each to 2.0 milliliters.

(iii) *Blank.* Use 2.0 milliliters of 0.1*N* hydrochloric acid in a 25-milliliter volumetric flask.

(iv) *Preparation of sample solutions.* Using a mortar and pestle, grind the sample to a fine powder. Transfer an accurately weighed portion of about 100 milligrams to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 1.0 milliliter to a 25-milliliter volumetric flask, add 1.0 milliliter of 0.1*N* hydrochloric acid, and mix.

(v) *Procedure.* To each of the flasks containing standards, blank, and sample, add 15 milliliters of hydrochloric acid-acetone solution and mix. Then add 3 milliliters of *p*- dimethylaminocinnamaldehyde solution to each and mix. Add 3 milliliters of 0.1*N* hydrochloric acid to each, dilute to volume with hydrochloric acid-acetone solution, mix well, and allow to stand at 25° C. for exactly 30 minutes. (Filter the sample solutions, if necessary, to remove any turbidity.) Using a suitable spectrophotometer, read the absorbance values of standard and sample solutions at a wavelength of 515 nanometers against the blank. Plot the absorbance values of the standards versus their concentrations and read the sample concentration from this standard response line.

(vi) *Calculations.*

$$\text{Percent hetacillin} = \frac{C \times 5{,}000 \times P}{\text{Weight of sample in milligrams}}$$

where:

C=Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

P=Hetacillin content of the hetacillin working standard in percent.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 44 FR 10379, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.29 Hetacillin potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Hetacillin potassium is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7.0 and not more than 9.0.

(v) Its hetacillin content is not less than 82 percent and not more than 95.5 percent.

(vi) It gives a positive identity test for hetacillin potassium.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Hetacillin content.* Proceed as directed in § 440.25(b)(5), except use about 110 milligrams of sample and calculate the hetacillin content as follows:

$$\text{Percent hetacillin} = \frac{C \times 5{,}000 \times P}{\text{Weight of sample in milligrams}}$$

where:

C=Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

P=Hetacillin content of the hetacillin working standard in percent.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 44 FR 10379, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.29a Sterile hetacillin potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin potassium is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram. If it is packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 1.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter (or when reconstituted as directed in the labeling, if it is packaged for dispensing) is not less than 7.0 and not more than 9.0.

(vii) Its hetacillin content is not less than 82 percent and not more than 95.5 percent.

(viii) It gives a positive identity test for hetacillin potassium.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers, except if each contains less than 450 milligrams, a minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient solution 3 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter using a solution containing the equivalent of 18 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter (or using a solution reconstituted as directed in the labeling, if it is packaged for dispensing).

(7) *Hetacillin content.* Proceed as directed in § 440.25(b)(5), except use about 110 milligrams of sample and calculate the potassium hetacillin content as follows:

$$\text{Percent hetacillin} = \frac{C \times 5{,}000 \times P}{\text{Weight of sample in milligrams}}$$

where:

C=Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

P=Hetacillin content of the hetacillin working standard in percent.

(8) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(9) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19876, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 43 FR 2393, Jan. 17, 1978; 44 FR 10379, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.36a Sterile methicillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methicillin sodium monohydrate is the monohydrated sodium salt of (2,6-dimethoxyphenyl) penicillin. It is so purified and dried that:

(i) It contains not less than 815 micrograms of methicillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 3 percent and not more than 6 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its methicillin content is not less than 81.5 percent.

(viii) It is crystalline.

(ix) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, methicillin content, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of methicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Sterility.* Proceed as directed in in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 60 milligrams of methicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(6) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Methicillin content.* Dissolve an accurately weighed portion of the sample in a sufficient accurately measured volume of distilled water to obtain a concentration of 0.2 milligram of methicillin per milliliter (estimated). Treat a portion of the methicillin working standard in the same manner. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and distilled water as the blank, determine the absorbance at 280 nanometers. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 250 nanometers to 300 nanometers. If a nonrecording spectrophotometer is used, determine the absorbance (on a solution containing 10 milligrams per 100 milliliters) at the 280-nanometer absorption peak. (The exact position of the peak should be determined for the particular instrument used.) Calculate as follows:

$$\text{Percent methicillin} = \frac{\text{Absorbance of sample} \times \text{weight of working standard} \times \text{volume of sample solution} \times \text{percent methicillin in working standard}}{\text{Absorbance of standard} \times \text{weight of sample} \times \text{volume of standard solution}}$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

(9) *Identity.* Using the sample solution prepared as described in paragraph (b)(7) of this section, determine the absorbancies at the absorption maximum at 280 nanometers and at the absorption minimum at 264 nanometers. The ratio of the two

$$A_{280}/A_{264}$$

should be not less than 1.30 and not more than 1.45.

[39 FR 18976, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975; 42 FR 59858, Nov. 22, 1977; 50 FR 19918, May 13, 1985]

§ 440.37a Sterile mezlocillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile mezlocillin sodium monohydrate is the monohydrate sodium salt of (2*S*, 5*R*, 6*R*)-3,3-dimethyl-6-[(*R*)-2-[3-(methylsulfonyl)-2-oxo-1-imidazolidine-carboxamido]-2-phenylacetamido]-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. It is so purified and dried that:

(i) It contains not less than 838 micrograms and not more than 978 micrograms of mezlocillin per milligram on an anhydrous basis. If it is packaged for dispensing, its mezlocillin content is not less than 90 percent and not more than 115 percent of the number of milligrams of mezlocillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.5 and not more than 8.0.

(vii) The specific rotation in an aqueous solution containing 10 milligrams of mezlocillin per milliliter at 25° C is 185°±10°.

(viii) It gives a positive identity test for mezlocillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, specific rotation, and identity.

(ii) Samples required:

(*a*) If it is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If it is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except:

(*a*) *Buffer.* In lieu of the buffer described in § 442.40(b)(1)(ii)(*b*)(*2*) of this chapter, use the buffer prepared as follows: Dissolve 200 grams of primary standard tris (hydroxymethyl) aminomethane in sufficient distilled water to make 1 liter. Filter before use.

(*b*) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the

mezlocillin working standard with sufficient distilled water to obtain a concentration of 2.0 milligrams of mezlocillin per milliliter.

(*c*) *Preparation of sample solution*. Dissolve an accurately weighed portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration; also, if packaged for dispensing, reconstitute as directed in the labeling using distilled water in lieu of the reconstituting fluid. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to a concentration of 2.0 milligrams of mezlocillin per milliliter (estimated).

(*d*) *Calculations*—(*1*) Calculate the mezlocillin content in micrograms per milligram as follows:

$$\text{Micrograms of mezlocillin per milligram of sample} = \frac{A_u \times P_a}{A_s \times W_u}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
W_u=Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the mezlocillin content of the single-dose vial as follows:

$$\text{Milligrams of mezlocillin per single-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
d=Dilution factor of the sample.

(*3*) Calculate the mezlocillin content of the multiple-dose vial as follows:

$$\text{Milligrams of mezlocillin per multiple-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
d=Dilution factor of the sample;
n=Volume of sample solution assayed.

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of mezlocillin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of mezlocillin per milliliter.

(7) *Specific rotation*. Dilute an accurately weighed sample with sufficient distilled water to obtain a concentration of approximately 10 milligrams of mezlocillin per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube.

(8) *Identity*. Proceed as directed in § 436.311 of this chapter, diluting the sample with distilled water to a concentration of 4 milligrams of mezlocillin per milliliter, except:

(i) Use the mezlocillin working standard and dilute with distilled water to a concentration of 4 milligrams of mezlocillin per milligram;

(ii) In lieu of the ninhydrin spray solution, after the plate is dried with a current of warm air, expose the plate to iodine vapors for about 30 seconds; and

(iii) Mezlocillin has an R_f value of about 0.67.

[46 FR 58298, Dec. 1, 1981, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.41 Nafcillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Nafcillin sodium monohydrate is the monohydrated sodium salt of 6-(2-ethoxy-1-naphthamido) penicillanic acid. It is so purified and dried that:

(i) It contains not less than 820 micrograms of nafcillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.5 percent and not more than 5.3 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 5.0 and not more than 7.0.

(v) It is crystalline.

(vi) Its nafcillin content is not less than 82.0 percent.

(vii) It gives a positive identity test for nafcillin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods: however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2 micrograms of nafcillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(5) *Crystallinity*. Proceed as directed in § 436.203(b) of this chapter.

(6) *Nafcillin content*. Dissolve an accurately weighed portion of the sample in a sufficient accurately measured volume of distilled water to obtain a concentration of 0.05 milligram of nafcillin per milliliter (estimated). Treat a portion of the nafcillin working standard in the same manner. Using a suitable spectrophotometer equipped with quartz cells and distilled water as a blank, scan the absorption spectra of the sample and the nafcillin working standard solutions between the wavelengths of 245 nanometers and 340 nanometers. Determine the absorbance of the sample and working standard solutions at the absorption maximum at 280±3 nanometers. (The exact position of the maximum should be determined for the particular instrument used.) Calculate as follows:

$$\text{Percent nafcillin} = \frac{\text{Absorbance of sample} \times \text{weight in milligrams of standard} \times \text{volume of sample solution} \times \text{nafcillin content of standard in percent}}{\text{Absorbance of standard} \times \text{weight in milligrams of sample} \times \text{volume of standard solution}}$$

(7) *Identity*. The absorption spectrum of the sample determined as directed in paragraph (b)(6) of this section compares qualitatively with that of the nafcillin working standard.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59858, Nov. 22, 1977; 46 FR 16683, Mar. 13, 1981; 50 FR 19918, 19919, May 13, 1985]

§ 440.41a Sterile nafcillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile nafcillin sodium monohydrate is the monohydrated sodium salt of 6-(2-ethoxy-1-naphthamido) penicillanic acid. It is so purified and dried that:

(i) It contains not less than 820 micrograms of nafcillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 3.5 nor more than 5.3 percent.

(vi) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 5.0 and not more than 7.0.

(vii) It is crystalline.

(viii) Its nafcillin content is not less than 82.0 percent.

(ix) It gives a positive identity test for nafcillin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, crystallinity, nafcillin content, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods: however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2 micrograms of nafcillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 80 milligrams of nafcillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(7) *Crystallinity.* Proceed as directed in § 436.203(b) of this chapter.

(8) *Nafcillin content.* Proceed as directed in § 440.41(b)(6).

(9) *Identity.* The absorption spectrum of the sample determined as directed in paragraph (b)(8) of this section compares qualitatively with that of the nafcillin working standard.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59858, Nov. 22, 1977; 45 FR 16474, Mar. 14, 1980; 45 FR 22921, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.49 Oxacillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxacillin sodium monohydrate is the monohydrated sodium salt of 5-methyl-3-phenyl-4-isoxazolyl penicillin. It is so purified and dried that:

(i) It contains not less than 815 and not more than 950 micrograms of oxacillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.5 and not more than 5.0 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 4.5 and not more than 7.5.

(v) Its oxacillin content is not less than 81.5 percent and not more than 95.0 percent.

(vi) It is crystalline.

(vii) It gives a positive identity test for the oxacillin moiety.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, oxacillin content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of oxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 30 milligrams per milliliter.

(5) *Oxacillin content.* Place approximately 60 milligrams of sample, accurately weighed, into a 100-milliliter volumetric flask. Dissolve and fill to volume with distilled water. Pipette a 5.0-milliliter aliquot of the sample solution into a 22- by 200-millimeter test tube, and add 5 milliliters of 10 *N* NaOH. Mix the solution, and place the tube in a boiling water bath for 60 minutes. Cool the tube, carefully add 10 milliliters of 6 *N* HCl, mix, and replace the tube in the boiling water bath for 10 minutes. Position the tube in the bath so that the liquid level in the tube is the same as the liquid level in the bath. After heating, remove the tube from the bath, carefully agitate the contents of the tube, and cool to room temperature. Quantitatively transfer the contents of the tube to a 250-milliliter volumetric flask. Add approximately 200 milliliters of freshly boiled and cooled distilled water, then 4.0 milliliters of 7.5 *N* NH_4OH, and dilute to volume with freshly boiled and cooled distilled water. Treat a sample of the oxacillin working standard in the same manner. Determine the absorbance of the sample and working standard solutions on a suitable spectrophotometer at 235 nanometers against a reagent blank, and calculate as follows:

$$\text{Percent oxacillin} = \frac{\text{Absorbance of sample} \times \text{Weight in milligrams of standard} \times \text{oxacillin content of standard in percent}}{\text{Absorbance of standard} \times \text{Weight in milligrams of sample}}$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Use the sample solution prepared as described in paragraph (b)(5) of this section and record the ultraviolet spectrum between 230 nanometers and 260 nanometers. It should be basically identical to that of the standard similarly treated.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59858, Nov. 22, 1977; 49 FR 5096, Feb. 10, 1984; 50 FR 19918, 19919, May 13, 1985]

§ 440.49a Sterile oxacillin sodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Sterile oxacillin sodium monohydrate is the monohydrated sodium salt of 5-methyl-3-phenyl-4-isoxazolyl penicillin. It is so purified and dried that:

(i) It contains not less than 815 and not more than 950 micrograms of oxacillin per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 3.5 and not more than 5.0 percent.

(vi) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 4.5 and not more than 7.5.

(vii) Its oxacillin content is not less than 81.5 percent and not more than 95.0 percent.

(viii) It is crystalline.

(ix) It gives a positive identity test for the oxacillin moiety.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, oxacillin content, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of oxacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of oxacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 30 milligrams per milliliter.

(7) *Oxacillin content.* Proceed as directed in § 440.49(b)(5).

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 440.49(b)(7).

[39 FR 18976, May 30, 1974, as amended at 42 FR 59858, Nov. 22, 1977; 50 FR 19918, 19919, May 13, 1985]

§ 440.55a Sterile penicillin G benzathine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G benzathine is the *N,N′*- dibenzylethylenediamine salt of penicillin G. It is so purified and dried that:

(i) Its potency is not less than 1,090 units and not more than 1,272 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 5.0 percent and not more than 8 percent.

(vi) Its pH in a 1:1 mixture of absolute ethyl alcohol and water containing 0.5 milligram per milliliter is not less than 4.0 and not more than 6.5.

(vii) Its penicillin G content is not less than 57.9 percent and not more than 71.6 percent.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, penicillin G content, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately measured representative portion of the sample in sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately, further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, medium F in lieu of medium E, and during the period of incubation shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, except prepare the sample as follows: Dissolve 50 milligrams of sample with 50 milliliters of absolute ethyl alcohol. Add 50 milliliters of distilled water and mix well.

(7) *Penicillin G content.* Accurately weigh approximately 50 milligrams of the sample, dissolve in absolute methyl alcohol, and dilute to 100 milliliters with absolute methyl alcohol. Treat a portion of the working standard in the same manner. Using a suitable spectrophotometer equipped with a quartz cell and absolute methyl alcohol as the blank, determine the absorbance at 263 nanometers. Calculate the percent penicillin G as follows:

$$\text{Percent penicillin G} = \frac{\text{Absorbance of sample} \times \text{weight in milligrams of standard} \times \text{percent penicillin G in standard}}{\text{Absorbance of standard} \times \text{weight in milligrams of sample}}$$

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59858, Nov. 22, 1977, as amended at 45 FR 16472, Mar. 14, 1980; 49 FR 6092, Feb. 17, 1984; 50 FR 19918, 19919, May 13, 1985]

§ 440.71 Penicillin V.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V is 3,3-dimethyl - 7-oxo-6-(2-phenoxyacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 1,525 units nor more than 1,780 units per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 2.0 percent.

(iv) Its pH in a saturated aqueous solution is not less than 2.5 and not more than 4.0.

(v) Its penicillin V content is not less than 90 percent and not more than 105 percent.

(vi) It is crystalline.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the statement ''For

use in the manufacture of nonparenteral drugs only.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, penicillin V content, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the bioassay method shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample (approximately 30 milligrams) in 2.0 milliliters of absolute methyl alcohol. Further dilute an aliquot of this solution with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution prepared by adding approximately 30 milligrams per milliliter.

(5) *Penicillin V content.* Accurately weigh approximately 20 milligrams of the sample, dissolve in absolute methanol, and make to 100 milliliters with absolute methyl alcohol. Treat a portion of the working standard in the same manner. Using a suitable spectrophotometer equipped with a quartz cell and absolute methyl alcohol as the blank, determine the absorbance of the peak at 276 nanometers. Calculate the percent penicillin V as follows:

$$\text{Percent penicillin V} = \frac{\text{Absorbance of sample} \times \text{weight in milligrams of standard} \times \text{Percent penicillin V in standard}}{\text{Absorbance of standard} \times \text{Weight in milligrams of sample}}$$

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59859, Nov. 22, 1977, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.73 Penicillin V potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V potassium is the potassium salt of 3,3-dimethyl-7-oxo-6-(2-phenoxyacetamido) - 4 -thia - 1 - azabicyclo[3.2.0]heptane - 2 - carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 1,380 units nor more than 1,610 units per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.5 percent.

(iv) Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 4.0 and not more than 7.5.

(v) Its penicillin V content is not less than 81.2 percent and not more than 94.7 percent.

(vi) It is crystalline.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the statement ''For use in the manufacture of nonparental drugs only.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, penicillin V content, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

(5) *Penicillin V content*. Dissolve and dilute approximately 20 milligrams of the sample, accurately weighed to 100 milliliters with 0.1*N* sodium hydroxide solution. Treat a portion of the penicillin V working standard in the same manner. Using a suitable spectrophotometer equipped with a quartz cell and 0.1*N* sodium hydroxide solution as the blank, determine the absorbance of the peak at 275 nanometers. Calculate the percent penicillin V as follows:

$$\text{Percent penicillin V} = \frac{\text{Absorbance of sample} \times \text{Weight in milligrams of standard} \times \text{Percent penicillin V in standard}}{\text{Absorbance of standard} \times \text{Weight in milligrams of sample}}$$

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59859, Nov. 22, 1977, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.74a Sterile penicillin G procaine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Penicillin G procaine is 3,3 - dimethyl - 7 - oxo - 6 - (2 - phenylacetamido) - 4 - thia - 1 - azabicyclo [3.2.0]heptane-2-carboxylic acid 2-(diethylamino) ethyl *p*-aminobenzoate compound (1:1). It is so purified and dried that:

(i) Its potency is not less than 900 units and not more than 1,050 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not less than 2.8 percent and not more than 4.2 percent.

(vi) Its pH in a saturated aqueous solution (about 300 milligrams per milliliter) is not less than 5.0 and not more than 7.5.

(vii) Its penicillin G content is not less than 51.0 percent and not more than 59.6 percent.

(viii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, penicillin G content, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase to diluting fluid A and swirl the flask to completely solubilize the sample before filtration. If the product contains lecithin, use diluting fluid D in lieu of A.

(3) *Pyrogens.* Proceed as directed in § 436.32(h) of this chapter, using a solution containing 2,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated solution prepared by suspending 300 milligrams of sample per milliliter.

(7) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59860, Nov. 22, 1977, as amended at 45 FR 16472, Mar. 14, 1980; 45 FR 22921, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.80 Penicillin G potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Penicillin G potassium is potassium 3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate. It is so purified and dried that:

(i) Its potency is not less than 1,440 units and not more than 1,680 units per milligram.

(ii) Its loss on drying is not more than 1.5 percent.

(iii) The pH of an aqueous solution containing 60 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(iv) Its penicillin G content is not less than 80.8 percent and not more than 94.3 percent.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on the batch for potency, loss on drying, pH, penicillin G content, and crystrallinity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Test and methods of assay*—(1) *Potency.* Proceed as directed in § 440.80a(b)(1).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

(4) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[55 FR 38674, Sept. 20, 1990]

§ 440.80a Sterile penicillin G potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G potassium is potassium 3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate. It is so purified and dried that:

(i) Its potency is not less than 1,440 units and not more than 1,680 units per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH in an aqueous solution containing 60 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its penicillin G content is not less than 80.8 percent and not more than 94.3 percent.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, penicillin G content, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

(7) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59860, Nov. 22, 1977, as amended at 45 FR 16472, Mar. 14, 1980; 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.81a Sterile penicillin G sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G sodium is sodium 3,3-dimethyl-7-oxo-6-(2 - phenylacetamido) - 4 - thia - 1 - azabicyclo [3.2.0] heptane-2-carboxylate. It is so purified and dried that:

(i) Its potency is not less than 1,500 units and not more than 1,750 units per milligram. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH in an aqueous solution containing 60 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its penicillin G content is not less than 84.5 percent and not more than 98.5 percent.

(viii) It is crystalline.

(ix) It passes the test for heat stability if it does not show a loss of more than 10 percent of its original potency.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, penicillin G content, crystallinity, and heat stability.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed portion of the sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative aliquot from each container. Dilute with solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

(7) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Heat stability.* Proceed as directed in § 436.214 of this chapter.

[42 FR 59861, Nov. 22, 1977, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.83a Sterile piperacillin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile piperacillin sodium is the sodium salt of (2*S*,5*R*, 6*R*)-6-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylate. It is so purified and dried that:

(i) Its potency is not less than 863 micrograms and not more than 1,007

micrograms of piperacillin per milligram on an anhydrous basis. If it is packaged for dispensing, it contains not less than 90.0 percent and not more than 120.0 percent of the number of grams of piperacillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 1.0 percent.

(vi) Its pH in an aqueous solution containing 400 milligrams per milliliter is not less than 5.5 and not more than 7.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) If it is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If it is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.334 of this chapter.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 150 milligrams of piperacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in §436.201 of this chapter, using the sample preparation method described in paragraph (d)(4) of that section.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 400 milligrams per milliliter

[47 FR 15769, Apr. 13, 1982, as amended at 50 FR 19918, 19919, May 13, 1985]

§440.90a Sterile ticarcillin disodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile ticarcillin disodium is 6-[(carboxy-3-thienylacetyl)] amino - 3, 3-dimethyl - 7 - oxo - 4 - thia - 1 - azabicyclo[3.2.0]heptane-2-carboxylic acid disodium salt. It is so purified and dried that:

(i) It contains not less than 800 micrograms of ticarcillin per milligram on an anhydrous basis. If it is packaged for dispensing, its ticarcillin content is not less than 90 percent and not more than 115 percent of the number of milligrams of ticarcillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of ticarcillin per milliliter (or if packaged for dispensing after reconstitution as directed in the labeling) is not less than 6.0 and not more than 8.0.

(vii) It gives a positive identity test for ticarcillin.

(viii) Its ticarcillin content is not less than 80 percent and not more than 94 percent on an anhydrous basis.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and ticarcillin content.

(ii) Samples required:

(*a*) If it is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams; and 5 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If it is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; and also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. If it is a single-dose container, use a separate needle and syringe for each container. Dilute with sufficient solution 1 to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of ticarcillin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of ticarcillin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ticarcillin per milliliter (or if packaged for dispensing, use a solution prepared as directed for reconstitution in the labeling).

(7) *Identity and ticarcillin content*. Transfer an accurately weighed portion of approximately 40 milligrams of the sample to a 100-milliliter volumetric flask. Dissolve and dilute to volume with distilled water. Transfer 5.0 milliliters of this solution to another 100-milliliter volumetric flask and dilute to volume with 0.1*N* methanolic hydrochloric acid (prepared by diluting 0.8 milliliter of 12*N* hydrochloric acid to 100 milliliters with methyl alcohol). Treat a portion of the ticarcillin standard in the same manner. Using a suitable spectrophotometer equipped with a 1.0-centimeter quartz cell and 0.1*N* methanolic acid as a blank, scan the absorption spectrum of the methanolic solution of the sample and the standard between the wavelengths of 300 and 200 nanometers. Determine the absorbance of each solution at the maxima, at approximately 230 nanometers. The spectrum of the samples should compare qualitatively with that of the ticarcillin working standard. Determine the percent ticarcillin as follows:

$$\text{Percent ticarcillin} = \frac{\text{Absorbance of sample} \times \text{Weight in milligrams of standard} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight in milligrams of sample} \times (100 - m)}$$

where: m=Percent moisture in the sample.

[42 FR 14093, Mar. 15, 1977, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.91 Ticarcillin monosodium monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ticarcillin monosodium monohydrate is 6-[(carboxy-3-thienylacetyl)] amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid monosodium salt monohydrate. It is so purified and dried that:

(i) Its ticarcillin potency is not less than 890 micrograms of ticarcillin per milligram calculated on an anhydrous basis.

(ii) Its moisture content is not less than 4.0 and not more than 6.0 percent.

(iii) The pH of an aqueous solution containing 10 milligrams of ticarcillin per milliliter is not less than 2.5 and not more than 4.0.

(iv) It gives a positive identity test for ticarcillin.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and crystallinity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Ticarcillin potency.* Determine the micrograms of ticarcillin activity per milligram of sample. Proceed as directed in § 436.355 of this chapter using the equipment, conditions, reagents, and system suitability requirements as described in § 440.290b(b), except use the resolution test solution to determine resolution in lieu of the working standard solution. Prepare the working standard solution, sample solution, and resolution test solution and calculate the micrograms of ticarcillin per milligrams as follows:

(i) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Accurately weigh a quantity of the ticarcillin working standard containing the equivalent of approximately 90 milligrams of ticarcillin activity and transfer to a 100-milliliter volumetric flask. Dissolve and dilute to volume with diluent pH 6.4 phosphate buffer prepared as described in § 440.290b(b)(1)(i)(*c*).

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample with diluent pH 6.4 buffer as prepared in § 440.290b(b)(1)(i)(*c*) to obtain a solution containing 0.9 milligram of ticarcillin activity per milliliter (estimated).

(C) *Resolution test solution.* Accurately weigh a quantity of the ticarcillin working standard containing the equivalent of approximately 90 milligrams of ticarcillin activity and transfer to a 100-milliliter volumetric flask. Prepare a solution of the clavulanic acid working standard containing the equivalent of 30 milligrams of clavulanic acid activity in a 100-milliliter volumetric flask. Dissolve and dilute to volume with diluent. Transfer 10 milliliters of this solution into the flask containing the ticarcillin standard. Dilute the combined standard solution to volume with diluent and mix. Use within 8 hours of preparation.

(ii) *Calculations.* Calculate the micrograms of ticarcillin per milligram as follows:

$$\text{Milligrams of amoxicillin or clavulanic acid per milliliter} = \frac{A_u \times C \times V \times 0.5}{A_s}$$

where:

A_u=Area of the ticarcillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ticarcillin peak in the chromatogram of the ticarcillin working standard;

P_s=Ticarcillin activity in the ticarcillin working standard solution in micrograms per milliliter;

C_u=Milligrams of ticarcillin sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ticarcillin per milliliter.

(4) *Identity.* Proceed as directed in § 440.90a(b)(7).

(5) *Crystallinity.* Proceed as directed in § 436.203 of this chapter.

[55 FR 5839, Feb. 20, 1990]

Subpart B—Oral Dosage Forms

§ 440.103 Amoxicillin oral dosage forms.

§ 440.103a Amoxicillin trihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate capsules are composed of amoxicillin trihydrate with or without one or more suitable and harmless lubricants, diluents, and drying agents, enclosed in a gelatin capsule. Each capsule contains amoxicillin trihydrate equivalent to 250 milligrams or 500 milligrams of amoxicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its moisture content is not more than 14.5 percent. It passes the identity test. The amoxicillin trihydrate used conforms to the standards prescribed by § 440.3(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''amoxicillin capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and identity.

(ii) Samples required:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive:

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of amoxicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with distilled water to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Identity.* Proceed as directed in § 436.311 of this chapter, preparing the sample solution as follows: Dissolve an accurately weighed portion of the amoxicillin capsule contents in 0.1*N* hydrochloric acid to give a solution containing 4 milligrams of amoxicillin per milliliter.

[39 FR 34033, Sept. 23, 1974, as amended at 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.103b Amoxicillin trihydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate for oral suspension is a mixture of amoxicillin trihydrate with one or more suitable and harmless colorings, flavorings, buffers, sweetening ingredients, preservatives, stabilizers, and suspending agents. When reconstituted as directed in the labeling, it contains amoxicillin trihydrate equivalent to either 25 or 50 milligrams of amoxicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its moisture content is not more than 3.0 percent. Its pH, when reconstituted as directed

in the labeling, is not less than 5.0 and not more than 7.5. It passes the identity test. The amoxicillin trihydrate used conforms to the standards prescribed by § 440.3(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''amoxicillin for oral suspension''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive:

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of amoxicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured aliquot (usually a single dose) into an appropriately sized volumetric flask and dilute to volume with distilled water. Mix well. Further dilute with distilled water to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension reconstituted as directed in the labeling.

(4) *Identity.* Proceed as directed in § 436.311 of this chapter, preparing the sample solution as follows: From an aliquot of suspension prepared in accordance with the label, make either a 6.25:1 dilution for the 25-milligrams-per-milliliter dosage; or a 12.5:1 dilution for the 50-milligrams-per-milliliter dosage, with 0.1*N* hydrochloric acid. The slight dilution of the acid does not have a significant effect on the test.

[39 FR 34033, Sept. 23, 1974, as amended at 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985; 54 FR 47351, Nov. 14, 1989]

§ 440.103c Amoxicillin trihydrate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate chewable tablets are composed of amoxicillin trihydrate with or without one or more suitable lubricants, diluents, preservatives, drying agents, flavorings, and colorings. Each tablet contains amoxicillin trihydrate equivalent to either 125 or 250 milligrams of amoxicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its moisture content is not more than 6.0 percent. It passes the identity test. The amoxicillin trihydrate used conforms to the standards prescribed by § 440.3(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by

§ 432.5 of this chapter, this drug shall be labeled ''amoxicillin tablets.''

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and identity.

(ii) Samples required:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with solution 3 to the reference concentration of 0.1 microgram of amoxicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 5 minutes. Further dilute with solution 1 to the prescribed concentration.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *Identity*. Proceed as directed in § 436.311 of this chapter, preparing the sample as follows: Using a mortar and pestle, grind a representative number of tablets into a fine powder. Dissolve an accurately weighed amount of this powder in 0.1*N* hydrochloric acid to give a solution containing 4 milligrams of amoxicillin per milliliter.

[45 FR 64569, Sept. 30, 1980, as amended at 50 FR 19919, May 13, 1985]

§ 440.103d Amoxicillin trihydrate and clavulanate potassium tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Amoxicillin trihydrate and clavulanate potassium tablets are composed of amoxicillin trihydrate and clavulanate potassium with or without one or more suitable lubricants, diluents, and binders. Each tablet contains amoxicillin trihydrate equivalent to either 250 or 500 milligrams of amoxicillin and clavulanate potassium equivalent to 125 milligrams of clavulanic acid. Its amoxicillin trihydrate content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of clavulanic acid that it is represented to contain. Its moisture content is not more than 7 percent if it contains 250 milligrams of amoxicillin and not more than 10 percent if it contains 500 milligrams of amoxicillin. It passes the dissolution test if the quantity *Q*, at 30 minutes, is 85 percent or greater if it contains 250 milligrams of amoxicillin and 75 percent or greater if it contains 500 milligrams of amoxicillin. The amoxicillin trihydrate conforms to the standards prescribed by § 440.3(a)(1). The clavulanate potassium conforms to the standards prescribed by § 455.15(a)(1) of this chapter.

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled *''amoxicillin and clavulanate potassium tablets''*.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The clavulanate potassium used in making the batch for clavulanic acid content, moisture, pH, identity, and clavam-2-carboxylate content.

(*c*) The batch for amoxicillin content, clavulanic acid content, moisture, and dissolution rate.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Amoxicillin and clavulanic acid contents.* Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirements, and calculations for amoxicillin or clavulanic acid content are as follows:

(i) *Reagents*—(*a*) *0.5M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4 ± 0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix methanol: 0.05*M* sodium phosphate buffer solution, pH 4.4 (5:95 v/v) and ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micron filter with vacuum. The mobile phase may be sparged with the helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Accurately weigh and transfer into a 200-milliliter volumetric flask approximately 100 milligrams of amoxicillin working standard and approximately 50 milligrams of the clavulanic acid working standard. Dissolve and dilute to volume with distilled water. Use within 8 hours after preparation.

(*b*) *Preparation of sample solution.* To obtain a concentration of 0.5 milligram of amoxicillin per milliliter, dissolve a representative number of tablets in water with the aid of a magnetic stirrer or ultrasonication. Filter a small aliquot through Whatman #42 filter paper or equivalent, discarding the first 10 milliliters of filtrate. Alternatively, a suitable membrane filter may be used. Prepare samples not more than 1 hour before the chromatographic injection.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 550 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the milligrams of amoxicillin or clavulanic acid content per tablet as follows:

$$\text{Milligrams of amoxicillin or clavulanic acid per tablet} = \frac{A_u \times C_s \times V}{A_s \times N}$$

where:

A_u=Response of the amoxicillin or clavulanic acid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Response of the amoxicillin or clavulanic acid peak in the chromatogram of the amoxicillin or clavulanic acid working standard;

C_s=Concentration of standards in milligrams of amoxicillin or clavulanic acid per milliliter of the standard solution;

V=Volume of sample solution (milliliters); and

N=Number of tablets taken for assay.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. Dissolution rate is determined by dissolution of the amoxicillin component using the high-performance liquid chromatographic assay described in this section.

[49 FR 39672, Oct. 10, 1984, as amended at 50 FR 19919, May 13, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.103e Amoxicillin trihydrate and clavulanate potassium for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate and clavulanate potassium for oral suspension is a dry mixture of amoxicillin trihydrate and clavulanate potassium with one or more suitable and harmless colorings, flavorings, buffers, sweetening ingredients, preservatives, stabilizers, and suspending agents. When reconstituted as directed in the labeling, each milliliter contains either amoxicillin trihydrate equivalent to 25 milligrams of amoxicillin with clavulanate potassium equivalent to 6.25 clavulanic acid or amoxicillin trihydrate equivalent to 50 milligrams of amoxicillin with clavulanate potassium equivalent to 12.5 milligrams of clavulanic acid. Its amoxicillin trihydrate content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of clavulanic acid that it is represented to contain. The moisture content of the dry powder is not more than 7.5 percent when the reconstituted solution is to contain 25 milligrams of amoxicillin per milliliter and not more than 8.5 percent when the reconstituted solution is to contain 50 milligrams of amoxicillin per milliliter. When reconstituted as directed in the labeling, its pH is not less than 4.8 and not more than 6.6. The amoxicillin trihydrate used conforms to the standards prescribed by § 440.3(a)(1). The clavulanate potassium conforms to the standards prescribed by § 455.15(a)(1) of this chapter.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled *''amoxicillin and clavulanate potassium for oral suspension''.*

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The clavulanate potassium used in making the batch for clavulanic acid content, moisture, pH, identity, and clavam-2-carboxylate content.

(*c*) The batch for amoxicillin content, clavulanic acid content, moisture, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Amoxicillin content or clavulanic acid content.* Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirments, and calculations for amoxicillin and clavulanic acid content are as follows:

(i) *Reagents*—(*a*) *0.05M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust to pH 4.4 ± 0.1 with 18*N*

phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix methanol:0.05*M* sodium phosphate buffer solution, pH 4.4 (5:95 v/v) and mix or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micron filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Accurately weigh and transfer into a 200-milliliter volumetric flask approximately 100 milligrams of amoxicillin working standard and approximately 50 milligrams of the clavulanate working standard. Dissolve and dilute to volume with distilled water. Use within 8 hours after preparation.

(*b*) *Preparation of sample solution.* Reconstitute the suspension as directed in the labeling. Immediately transfer an appropriate aliquot to a suitable volumetric flask to obtain an approximate amoxicillin concentration of 0.5 milligram per milliliter and dilute to volume with distilled water. Mix well for 10 minutes using a magnetic stirrer. Filter an aliquot through Whatman #42 or equivalent filter paper. Alternatively, a suitable membrane filter may be used. Samples should be prepared just prior to chromatographic injection. Inject the sample solution within 1 hour after the addition of water.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 550 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the quantity of amoxicillin or clavulanic acid content in milligrams per milliliter of the oral suspension as follows:

$$\text{Milligrams of amoxicillin or clavulanic acid per milliliter} = \frac{A_u \times C \times V \times 0.5}{A_s}$$

where:

A_u=Response of the amoxicillin or clavulanic acid peaks in the sample chromatogram;

A_s=Response of the amoxicillin or clavulanic acid peaks in the standard chromatogram;

C=Concentration of the standard (milligrams per milliliter of amoxicillin X potency of amoxicillin standard or milligrams per milliliter of clavulanate X potency of clavulanate standard); and

V=Dilution volume in milliliters.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension reconstituted as directed in the labeling.

[49 FR 39673, Oct. 10, 1984, as amended at 50 FR 19919, May 13, 1985; 55 FR 11582, Mar. 29, 1990]

§ 440.103f Amoxicillin trihydrate-clavulanate potassium chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amoxicillin trihydrate-clavulanate potassium chewable tablets are composed of amoxicillin trihydrate and clavulanate potassium with or without one or more suitable lubricants, diluents, flavorings, and binders. Each tablet contains amoxicillin trihydrate equivalent to either 125 or 250 milligrams of amoxicillin and clavulanate potassium equivalent to 31.25 or 62.5 milligrams of clavulanic acid. Its amoxicillin trihydrate content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of amoxicillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 90 percent and not more than 120 percent

of the number of milligrams of clavulanic acid that it is represented to contain. Its moisture content is not more than 6 percent. It passes the dissolution test if the quantity *Q*, of amoxicillin at 30 minutes, is 85 percent or greater. The amoxicillin trihydrate conforms to the standards prescribed by § 440.3(a)(1). The clavulanate potassium conforms to the standards prescribed by § 455.15(a)(1) of this chapter.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled *''amoxicillin-clavulanate potassium chewable tablets''.*

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amoxicillin trihydrate used in making the batch for potency, safety, moisture, pH, amoxicillin content, concordance, crystallinity, and identity.

(*b*) The clavulanate potassium used in making the batch for clavulanic acid content, moisture, pH, identity, and clavam-2-carboxylate content.

(*c*) The batch for amoxicillin content, clavulanic acid content, moisture, and dissolution rate.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The amoxicillin trihydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Amoxicillin and clavulanic acid contents.* Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas. Reagents, working standard and sample solutions, system suitability requirements, and calculations for amoxicillin or clavulanic acid content are as follows:

(i) *Reagents*—(*a*) *0.05M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of sodium monobasic phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4±0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix methanol: 0.05*M* sodium phosphate buffer solution, pH 4.4 (5:95 v/v) and ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micron filter with vacuum. The mobile phase may be sparged with the helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Working standard and sample solutions—(a) Preparation of working standard solution.* Dissolve and dilute accurately weighed portions each of the amoxicillin trihydrate working standard and the clavulanate lithium working standard with water to obtain a solution containing 0.5 milligram of amoxicillin and 0.25 milligram of clavulanic acid per milliliter. Use within 1 hour after preparation or within 4 hours if stored under refrigeration.

(*b*) *Preparation of sample solution.* To obtain a concentration of 0.5 milligram of amoxicillin per milliliter, dissolve a representative number of tablets in water with the aid of a magnetic stirrer or ultrasonication. Filter an aliquot through Whatman #42 filter paper or equivalent, discard the first 10 milliliters of filtrate, and use the remaining portion as the sample solution. Alternatively, a suitable membrane filter may be used. Prepare samples not more than 1 hour before the chromatographic injection.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates in a 30-centimeter column for each active component.

(*c*) *Resolution.* The resolution (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the milligrams of amoxicillin or clavulanic acid content per tablet as follows:

$$\text{Milligrams of amoxicillin or clavulanic acid per tablet} = \frac{A_u \times C_s \times V}{A_s \times N}$$

where

A_u=Response of the amoxicillin or clavulanic acid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Response of the amoxicillin or clavulanic acid peak in the chromatogram of the amoxicillin or clavulanic acid working standard;

C_s=Concentration of standards in milligrams of amoxicillin or clavulanic acid per milliliter of the standard solution;

V=Volume of sample solution (milliliters); and

N=Number of tablets taken for assay.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. Dissolution rate is determined by dissolution of the amoxicillin component using the high-performance liquid chromatographic assay described in this section.

[50 FR 42933, Oct. 25, 1985; 50 FR 47367, Nov. 17, 1985, as amended at 55 FR 11582, Mar. 29, 1990]

§ 440.105 Ampicillin oral dosage forms.

§ 440.105a Ampicillin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin tablets are composed of ampicillin with one or more suitable and harmless diluents and lubricants. Each tablet contains 250 or 500 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not more than 4 percent. The tablets disintegrate within 15 minutes. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place a representative number of tablets in a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.105b Ampicillin chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each ampicillin chewable tablet contains 125 milligrams or 250 milligrams of ampicillin with suitable binders, lubricants, flavorings, and colorings. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not more than 3 percent. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''ampicillin tablets''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) The results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Blend a representative number of tablets in a high-speed blender with sufficient distilled water to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 43 FR 9800, Mar. 10, 1978; 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.105c Ampicillin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin capsules are composed of ampicillin with or without one or more buffer substances, diluents, binders, lubricants, vegetable oils, colorings, and flavorings, enclosed in a gelatin capsule. Each capsule contains 125 milligrams, 250 milligrams, or 500 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The loss on drying is not more than 4.0 percent. The ampicillin used conforms to the standards prescribed by § 440.5(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in §436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Filter through Whatman No. 2 filter paper. Further dilute an aliquot of the filtrate with distilled water to the prescribed concentration.

(2) *Loss on drying*. Proceed as directed in §436.200(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§440.105d Ampicillin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin for oral suspension is a mixture of ampicillin with one or more suitable and harmless colorings, flavorings, buffer substances, sweetening ingredients, and preservatives. When reconstituted as directed in the labeling, it contains either 25 milligrams, 50 milligrams, or 100 milligrams of ampicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 2.5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The ampicillin used conforms to the standards prescribed by §440.5(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in §436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as

follows: Reconstitute the drug as directed in the labeling. Place an accurately measured aliquot (usually a single dose) into an appropriately sized volumetric flask and dilute to volume with distilled water. Mix well. Further dilute with distilled water to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3458, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.107 Ampicillin trihydrate oral dosage forms.

§ 440.107a Ampicillin trihydrate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate chewable tablets are composed of ampicillin trihydrate with or without one or more suitable diluents, lubricants, preservatives, and flavorings. Each tablet contains ampicillin trihydrate equivalent to 125 or 250 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 5.0 percent. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''ampicillin tablets.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient distilled water to give a convenient concentration. Blend for 5 minutes. Further dilute with distilled water to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3459, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.107b Ampicillin trihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate capsules are composed of ampicillin trihydrate with or without one or more buffer substances, diluents, binders, lubricants, vegetable oils, colorings, and flavorings enclosed in a gelatin capsule. Each capsule contains ampicillin trihydrate equivalent to 250 milligrams or 500 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than

120 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not less than 10 percent and not more than 15 percent. The ampicillin trihydrate used conforms to the standards prescribed by §440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by §432.5 of this chapter, this drug shall be labeled ''ampicillin capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in §436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Filter through Whatman No. 2 filter paper. Further dilute an aliquot of the filtrate with distilled water to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in §436.200(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3459, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§440.107c Ampicillin trihydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin trihydrate for oral suspension is a mixture of ampicillin trihydrate with one or more suitable and harmless colorings, flavorings, buffers, sweetening ingredients, and preservatives. When reconstituted as directed in the labeling, it contains ampicillin trihydrate equivalent to either 25, 50, or 100 milligrams of ampicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is:

(i) Not more than 2.5 percent if it contains sugar and is intended to contain the equivalent of 25 or 50 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling; or

(ii) Not more than 5 percent if it contains sugar and is intended to contain the equivalent of 100 milligrams of ampicillin per milliliter when reconstituted as directed in the labeling; or

Its pH, when reconstituted as directed in the labeling, is not less than 5.0 and is not more than 7.5. The ampicillin trihydrate used conforms to the standards prescribed by §440.7(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by §432.5 of this chapter, this drug shall be labeled ''ampicillin for oral suspension.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on

drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured aliquot (usually a single dose) into an appropriately sized volumetric flask and dilute to volume with distilled water. Mix well. Further dilute with distilled water to the prescribed concentration.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3459, Jan. 27, 1984; 49 FR 5096, Feb. 10, 1984; 50 FR 19919, May 13, 1985]

§ 440.107d Ampicillin trihydrate-probenecid for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin trihydrate and probenecid for oral suspension is a dry mixture of ampicillin trihydrate and probenecid with suitable flavorings, lubricants, colorings, and suspending agents packaged in a single-dose container. When reconstituted as directed in the labeling, each single dose will contain ampicillin trihydrate equivalent to 3.5 grams of ampicillin and 1.0 gram of probenecid. Its ampicillin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of grams of ampicillin that it is represented to contain. Its probenecid content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of grams of probenecid that it is represented to contain. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1). The probenecid used conforms to the standards prescribed by the U.S.P.

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''ampicillin-probenecid for oral suspension''.

(3) *Requests for certification, samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The probenecid used in making the batch for all U.S.P. specifications.

(*c*) The batch for ampicillin content, probenecid content, moisture, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Ampicillin content*—(i) *Sample preparation*. Reconstitute as directed in the labeling and mix well. Drain the suspension from the bottle for 30 seconds into a high-speed glass blender jar containing

sufficient sterile distilled water to obtain a total volume of 500 milliliters. Blend for 10 minutes.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the aqueous solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Dilute an aliquot of the aqueous solution to the prescribed concentration.

(2) *Probenecid content*—(i) *Preparation of standard solution*. Transfer approximately 25 milligrams of U.S.P. probenecid reference standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with 1 percent aqueous sodium carbonate solution.

(ii) *Preparation of sample solution*. Reconstitute the sample as directed in the labeling and mix well. Drain the suspension from the bottle for 30 seconds into a 1,000-milliliter volumetric flask. Dilute to volume with 1 percent aqueous sodium carbonate solution, shake well, and filter through Whatman No. 6 filter paper. Discard the first 10-milliliter portion.

(iii) *Procedure.* Transfer 2.0 milliliters of the clear filtrate to a 125-milliliter separatory funnel and add 8.0 milliliters of 1.0*N* hydrochloric acid. Extract the solution with four 20-milliliter portions of chloroform, filtering each extract through a glass wool pledget into a 100-milliliter volumetric flask. Wash the pledget with chloroform, dilute to volume with chloroform and mix. Treat 2.0 milliliters of the standard solution in the same manner. Using a suitable spectrophotometer equipped with a 1-centimeter cell and chloroform washed with 1 percent aqueous sodium carbonate solution as a blank, determine the absorbance of the sample and standard solutions at the peak near 257 nanometers.

(iv) *Calculations.* Calculate the probenecid content as follows:

Grams probenecid per container=(Absorbance of sample×weight of standard in milligrams×percent purity of standard)/(Absorbance of standard×25×100)

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 40 FR 49083, Oct. 21, 1975; 49 FR 3459, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.107e Ampicillin trihydrate-probenecid capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ampicillin trihydrate-probenecid capsules are composed of ampicillin trihydrate and probenecid with or without one or more buffer substances, diluents, binders, lubricants, vegetable oils, colorings, and flavorings enclosed in a gelatin capsule. Each capsule contains ampicillin trihydrate equivalent to 389 milligrams of ampicillin and 111 milligrams of probenecid. Its ampicillin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its probenecid content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of probenecid that it is represented to contain. Its loss on drying is not less than 8.5 percent and not more than 13.0 percent. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7(a)(1). The probenecid used conforms to the standards prescribed by the U.S.P.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''ampicillin-probenecid capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The probenecid used in making the batch for all U.S.P. specifications.

(*c*) The batch for ampicillin content, probenecid content, and loss on drying.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Ampicillin content.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 8 to 10 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Prepare the sample as follows: Place the contents of a representative number of capsules into a high-speed glass blender jar with sufficient distilled water to give a convenient concentration. Blend for 8 to 10 minutes. Filter through Whatman No. 2 filter paper. Further dilute an aliquot of the filtrate with distilled water to the prescribed concentration.

(2) *Probenecid content*—(i) *Preparation of standard solution.* Transfer approximately 25 milligrams of probenecid reference standard U.S.P., accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with 1 percent aqueous sodium carbonate solution.

(ii) *Preparation of sample solution.* Place the contents of a representative number of capsules into a high-speed glass blender jar with 100 milliliters of 1 percent aqueous sodium carbonate solution for each capsule. Blend for 8 to 10 minutes. Filter a portion through Whatman No. 2 filter paper, discarding the first 10-milliliter portion of the filtrate.

(iii) *Procedure.* Transfer 2.0 milliliters of the clear filtrate to a 125-milliliter separatory funnel and add 8.0 milliliters of 1.0*N* hydrochloric acid. Extract the solution with four 20-milliliter portions of chloroform, filtering each extract into a 100-milliliter volumetric flask through a glass wool pledget and 6 grams of chloroform-washed anhydrous sodium sulfate. Wash the pledget and sodium sulfate with chloroform, dilute to volume with chloroform and mix. Treat 2.0 milliliters of the standard solution in the same manner. Using a suitable spectrophotometer equipped with a 1-centimeter cell and chloroform washed with 1 percent aqueous sodium carbonate solution as a blank, determine the absorbance of the sample and standard solutions at the peak near 257 nanometers.

(iv) *Calculations.* Calculate the probenecid content as follows:

Milligrams probenecid per capsule = (Absorbance of sample × weight of standard in milligrams × percent purity of standard)/(Absorbance of standard × 25)

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

[40 FR 58288, Dec. 16, 1975, as amended at 45 FR 16474, Mar. 14, 1980; 49 FR 3459, Jan. 27, 1984; 50 FR 19919, May 13, 1985]

§ 440.108 Bacampicillin hydrochloride dosage forms.

§ 440.108a Bacampicillin hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacampicillin hydrochloride tablets are composed of bacampicillin hydrochloride with one or more suitable and harmless diluents and lubricants. Each tablet contains bacampicillin hydrochloride equivalent to either 280 or 560 milligrams of ampicillin. Its potency is satisfactory if it is not less than 90 percent and not more

than 125 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 2.5 percent. It passes the dissolution test. The bacampicillin hydrochloride used conforms to the standards prescribed by §440.8(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacampicillin hydrochloride used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and dissolution.

(ii) Samples required:

(*a*) The bacampicillin hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Hydroxylamine colorimetric assay.* Proceed as directed in §440.8(b)(1)(i) of this chapter, except prepare the sample solution and calculate the potency of the sample as follows:

(*a*) *Preparation of sample solution.* Place one tablet into a high-speed glass blender jar with sufficient distilled water to obtain a concentration of 1.25 milligrams of ampicillin per milliliter (estimated). Blend for 3 to 5 minutes. Filter before using.

(*b*) *Calculations.* Calculate the ampicillin content in milligrams per tablet as follows:

$$\text{Milligrams of cyclacillin per tablet} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;

P_a=Potency of working standard in micrograms per milliliter;

A_s=Absorbance of working standard solution;

d=Dilution factor of the sample.

(ii) *Iodometric assay.* Proceed as directed in §436.204 of this chapter, except use the ampicillin working standard. Prepare the sample as follows: Dissolve and dilute a representative number of tablets with distilled water to the prescribed concentration.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in §436.215 of this chapter, except in lieu of paragraph (d) of that section use the interpretation described in the United States Pharmacopeia XX dissolution test. The quantity, *Q* (the amount of ampicillin dissolved) is 85 percent at 30 minutes.

[46 FR 25604, May 8, 1981. Redesignated at 47 FR 23711, June 1, 1982, and amended at 48 FR 51293, 51294, Nov. 8, 1983; 50 FR 19919, May 13, 1985]

§440.108b Bacampicillin hydrochloride for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacampicillin hydrochloride for oral suspension is a mixture of bacampicillin hydrochloride with one or more suitable and harmless buffers, diluents, sweetening ingredients, suspending agents, flavorings, and colorings. When reconstituted as directed in the labeling, it contains bacampicillin hydrochloride equivalent to 17.5 milligrams of ampicillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of ampicillin that it is represented to contain. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.5 and not more than 8.0. It gives a positive identity test for bacampicillin hydrochloride. The bacampicillin hydrochloride conforms to the standards prescribed by §440.8(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacampicillin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, loss on drying, pH, and identity.

(ii) Samples required:

(*a*) The bacampicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.204 of this chapter, except:

(i) Use the ampicillin working standard as the standard of comparison;

(ii) Use 4.0 milliliters of sample solution in lieu of the 2.0 milliliters specified in paragraph (c)(1) of that section; and

(iii) Calculate the potency of the sample as follows:

$$\text{Milligrams of ampicillin per dose} = \frac{V_u \times F \times d}{n \times 4{,}000}$$

Prepare the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured portion equivalent to one dose into a 250-milliliter volumetric flask. Add 200 milliliters of a solvent mixture of 95 percent ethanol and 0.1*M* phosphoric acid (8:2). Shake for 30 minutes on a wrist action shaker and dilute to volume with the solvent mixture. Centrifuge a portion of the sample solution for 10 minutes at 6,000 rpm. Use the clear supernatant without further dilution.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(4) *Identity*. Proceed as directed in § 436.330 of this chapter, except prepare the sample as follows: Reconstitute as directed in the labeling. Place 8.0 milliliters of the sample into a 100-milliliter volumetric flask, add 70 milliliters of 95 percent ethyl alcohol and shake for 30 minutes. Dilute to volume with 95 percent ethyl alcohol.

[47 FR 23711, June 1, 1982, as amended at 50 FR 19919, May 13, 1985]

§ 440.111 Carbenicillin indanyl sodium tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Carbenicillin indanyl sodium tablets are composed of carbenicillin indanyl sodium and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains carbenicillin indanyl sodium equivalent to 382 milligrams of carbenicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of carbenicillin that it is represented to contain. Its moisture content is not more than 2.0 percent. It gives a positive identity test for carbenicillin indanyl sodium. The tablets shall disintegrate within 1 hour. The carbenicillin indanyl sodium used conforms to the standards prescribed by § 440.11(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The carbenicillin indanyl sodium used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, moisture, identity, and disintegration time.

(ii) Samples required:

(*a*) The carbenicillin indanyl sodium used in making the batch: Five packages, each containing approximately 1 gram and one package containing approximately 2.5 grams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.300 of this chapter, except:

(i) *Preparation of the sample.* Accurately weigh 20 tablets and determine the average tablet weight. Using a mortar and pestle, grind the tablets to a fine powder. Accurately weigh a portion of the powder approximately equivalent to the weight of one tablet and transfer it into a 100-milliliter volumetric flask. Add approximately 70 milliliters of distilled water and shake

the flask for 5 minutes. Dilute to volume and mix well. Transfer a 5-milliliter aliquot of the stock solution to a 50-milliliter glass-stoppered centrifuge tube. (The solution will be slightly turbid.) Add 15 milliliters of phosphate-citrate buffer and 20 milliliters of 4-methyl-2-pentanone to the tube. Stopper the tube and shake it for 10 seconds. Centrifuge at 2,000 revolutions per minute to separate the phases. Remove about 15 milliliters of the upper phase and proceed as directed in §436.300(e) of this chapter.

(ii) *Calculations.* Calculate the carbenicillin content (potency) of the tablets as follows:

Milligrams of carbenicillin per tablet=(Degrees of rotation of sample solution × weight of working standard × average tablet weight × 100 × micrograms of carbenicillin in each milligram of the working standard)/(Degrees of rotation of working standard×weight of sample × 25 × 1,000)

where:

100 and 25=The volume of the sample and working standard solutions, respectively;

1,000=Factor to correct micrograms to milligrams.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Identity.* Proceed as directed in §436.301 of this chapter, preparing the sample as follows: Using a mortar and pestle, grind a representative number of tablets into a fine powder. Dissolve a weighed amount of this powder in sufficient extraction solvent (described in §436.301(b)(1) of this chapter) to give 10 milligrams of carbenicillin per milliliter. Shake the mixture for 5 minutes and promptly dilute an aliquot in extraction solvent to obtain a final concentration of 1 milligram carbenicillin per milliliter.

(4) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

[39 FR 18976, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§440.115 Cloxacillin sodium monohydrate oral dosage forms.

§440.115a Cloxacillin sodium monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cloxacillin sodium monohydrate capsules are composed of cloxacillin sodium and one or more suitable and harmless diluents and lubricants. Each capsule contains cloxacillin sodium monohydrate equivalent to 125 milligrams, 250 milligrams, or 500 milligrams of cloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cloxacillin that it is represented to contain. Its moisture content is not more than 5 percent. The cloxacillin sodium monohydrate used conforms to the standards prescribed by §440.15(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''cloxacillin sodium capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cloxacillin sodium monohydrate used in making the batch for potency, moisture, pH, cloxacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cloxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59861, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.115b Cloxacillin sodium monohydrate for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cloxacillin sodium monohydrate for oral solution is a mixture of sodium cloxacillin with one or more suitable and harmless colorings, flavorings, buffer substances, and preservatives. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 25 milligrams or 50 milligrams of cloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cloxacillin that it is represented to contain. Its moisture content is not more than 1 percent. When reconstituted as directed in its labeling, its pH is not less than 5.0 nor more than 7.5. The cloxacillin sodium monohydrate used conforms to the standards prescribed by § 440.15(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''cloxacillin sodium for oral solution''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cloxacillin sodium monohydrate used in making the batch for potency, moisture, pH, cloxacillin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The cloxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute the sample as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of cloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(3) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in its labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59861, Nov. 22, 1977; 43 FR 9800, Mar. 10, 1978; 50 FR 19919, May 13, 1985]

§ 440.117 Cyclacillin oral dosage forms.

§ 440.117a Cyclacillin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclacillin tablets are composed of cyclacillin with one or more suitable and harmless diluents, lubricants, colorings, and disintegrants. Each tablet contains 250 or 500 milligrams of cyclacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of

the number of milligrams of cyclacillin that it is represented to contain. Its moisture content is not more than 5 percent. The tablets disintegrate within 15 minutes. It gives a positive identity test for cyclacillin. The cyclacillin used conforms to the standards prescribed by § 440.17(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cyclacillin used in making the batch for potency, moisture, pH, cyclacillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, disintegration time, and identity.

(ii) Samples required:

(*a*) The cyclacillin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of cyclacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample solution as follows: Place a representative number of tablets in a high-speed glass blender jar and add sufficient distilled water to give a convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot with distilled water to the prescribed concentration.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(*a*) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cyclacillin working standard in sufficient distilled water to obtain a concentration of 1.25 milligrams of cyclacillin per milliliter.

(*b*) *Preparation of sample solution.* Place one tablet into a high-speed glass blender jar and add sufficient distilled water to obtain a concentration of 1.25 milligrams of cyclacillin per milliliter. Blend for 3 to 5 minutes. Filter, if necessary.

(*c*) *Calculations.* Calculate the cyclacillin content in milligrams per tablet as follows:

$$\text{Milligrams of cyclacillin per 5 milliliters of sample} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;

P_a=Potency of working standard in micrograms per milliliter;

A_s=Absorbance of working standard solution;

d=Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure in paragraph (e)(1) of that section, except do not use discs.

(4) *Identity.* Proceed as directed in § 436.327 of this chapter, preparing the sample as follows: Dissolve a representative portion of finely powdered tablets with sufficient 0.1*N* sodium hydroxide to obtain a solution containing 1 milligram of cyclacillin per milliliter. Allow the sample solution to stand for 15 minutes before using.

[46 FR 2985, Jan. 13, 1981; 46 FR 15880, Mar. 10, 1981, as amended at 50 FR 19919, May 13, 1985]

§ 440.117b Cyclacillin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclacillin for oral suspension is a mixture of cyclacillin with one or more suitable and harmless colorings, flavorings, buffer substances, sweetening ingredients, preservatives,

and suspending agents. When reconstituted as directed in the labeling, it contains either 25 milligrams, 50 milligrams, or 100 milligrams of cyclacillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cyclacillin that it is represented to contain. Its moisture content is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.5. It gives a positive identity test for cyclacillin. The cyclacillin used conforms to the standards prescribed by § 440.17(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cyclacillin used in making the batch for potency, moisture, pH, cyclacillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The cyclacillin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into a suitable volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a convenient concentration. Mix well. Further dilute an aliquot with solution 3 to the reference concentration of 1.0 microgram of cyclacillin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute the drug as directed in the labeling. Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Mix well. Further dilute with solution 1 to the prescribed concentration.

(iii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(*a*) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cyclacillin working standard in sufficient distilled water to obtain a concentration of 1.25 milligrams of cyclacillin per milliliter.

(*b*) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling. Place an accurately measured aliquot of the sample into an appropriate-sized volumetric flask and dilute to volume with distilled water to yield a concentration of 1.25 milligrams of cyclacillin per milliliter. Mix well. Filter, if necessary.

(*c*) *Calculations.* Calculate the cyclacillin content as follows:

$$\begin{array}{c}\text{Milligrams of}\\ \text{cyclacillin per}\\ \text{5 milliliters of sample}\end{array} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;

P_a=Potency of working standard in micrograms per milliliter;

A_s=Absorbance of working standard solution;

d=Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(4) *Identity.* Proceed as directed in § 436.327 of this chapter, preparing the sample as follows: Dilute an accurately measured representative portion of the reconstituted suspension with 0.1*N* sodium hydroxide to obtain a solution containing 1 milligram of cyclacillin

per milliliter. Allow the sample solution to stand 45 minutes before using.

[46 FR 2985, Jan. 13, 1981; 46 FR 15880, Mar. 10, 1981, as amended at 50 FR 19919, May 13, 1985]

§ 440.119 Dicloxacillin sodium monohydrate oral dosage forms.

§ 440.119a Dicloxacillin sodium monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dicloxacillin sodium monohydrate capsules are composed of dicloxacillin sodium monohydrate and one or more suitable diluents and lubricants. Each capsule contains dicloxacillin sodium monohydrate equivalent to 62.5, 125, 250, or 500 milligrams of dicloxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dicloxacillin that it is represented to contain. The moisture content is not more than 5 percent. The dicloxacillin sodium monohydrate conforms to the requirements of § 440.19(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''dicloxacillin sodium capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The dicloxacillin sodium monohydrate used in making the batch for potency, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The dicloxacillin sodium monohydrate used in making the batch: 10 containers, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of dicloxacillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedure.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59861, Nov. 22, 1977; 43 FR 2393, Jan. 17, 1978; 44 FR 10379, Feb. 20, 1979; 50 FR 19919, May 13, 1985]

§ 440.119b Dicloxacillin sodium monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dicloxacillin sodium monohydrate for oral suspension is a mixture of dicloxacillin sodium monohydrate with one or more suitable colorings, flavorings, buffer substances, and preservatives. When reconstituted as directed in the labeling, it contains the equivalent of 12.5 or 25 milligrams of dicloxacillin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dicloxacillin that it is represented to contain. Its moisture content is not more than 2 percent. The pH of the suspension, when reconstituted as directed in the labeling, is not less than 4.5 nor more than 7.5. The dicloxacillin sodium monohydrate used conforms to the requirements of § 440.19(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''dicloxacillin sodium for oral suspension''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The dicloxacillin sodium monohydrate used in making the batch for potency, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The dicloxacillin sodium monohydrate used in making the batch: 10 containers, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute the sample as directed in the labeling. Place an accurately measured aliquot of the sample containing an estimated 125 milligrams of dicloxacillin into a 100-milliliter volumetric flask. Add 20 milliliters of dimethylformamide and shake mechanically for 30 minutes. Dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). The addition of dimethylformamide may be omitted if complete solution can be obtained with solution 1. Further dilute an aliquot with sufficient solution 1 to the reference concentration of 5.0 micrograms of dicloxacillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedure.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59861, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.125 Hetacillin oral dosage forms.

§ 440.125a Hetacillin chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each hetacillin chewable tablet contains an amount of hetacillin equivalent to 112.5 milligrams of ampicillin with suitable buffers, preservatives, binders, flavorings, colorings, and sweetening ingredients. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The moisture content is not more than 2.0 percent. The hetacillin used conforms to the requirements of § 440.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin used in making the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required.

(*a*) The hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Place a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 440.125b Hetacillin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin for oral suspension is a mixture of hetacillin with one or more suitable preservatives, suspending agents, sweetening ingredients, flavorings, and colorings. When reconstituted as directed in the labeling, it contains the equivalent of 22.5, 45, or 112.5 milligrams of ampicillin per milliliter. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. Its moisture content is not more than 2.0 percent. The pH of the suspension, when reconstituted as directed in its labeling, is not less than 2.0 and not more than 5.0. The hetacillin used conforms to the requirements of § 440.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin used in making the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The hetacillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Remove an accurately measured representative portion with a suitable syringe and hypodermic needle and place into a suitable volumetric flask. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3). Further dilute an aliquot with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample after reconstituting as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 440.129 Hetacillin potassium capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin potassium capsules are composed of potassium hetacillin with or without one or more suitable diluents, lubricants, and drying agents. Each capsule contains an amount of potassium hetacillin equivalent to 112.5, 225, or 450 milligrams of ampicillin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. The moisture content is not more than 3 percent. The potassium hetacillin used conforms to the requirements of § 440.29(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin potassium used in making the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The hetacillin potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing

the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59861, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.141 Nafcillin sodium monohydrate oral dosage forms.

§ 440.141a Nafcillin sodium monohydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nafcillin sodium monohydrate tablets are composed of nafcillin sodium monohydrate with one or more suitable buffers, binders, disintegrants, diluents, and lubricants. Each tablet contains nafcillin sodium monohydrate equivalent to 500 milligrams of nafcillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. Its moisture content is not more than 5 percent. It shall disintegrate within 20 minutes. The nafcillin sodium monohydrate used conforms to the standards prescribed by § 440.41(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''nafcillin sodium tablets''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nafcillin sodium monohydrate used in making the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(1) of that section, except use distilled water in lieu of simulated gastric fluid as the immersion fluid.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59862, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.141b Nafcillin sodium monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nafcillin sodium monohydrate capsules are composed of nafcillin sodium monohydrate and one or more suitable and harmless buffer substances and lubricants. Each capsule contains nafcillin sodium monohydrate equivalent to 250 milligrams of nafcillin. The potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin

that it is represented to contain. The moisture content is not more than 5.0 percent. The nafcillin sodium monohydrate conforms to the standards prescribed by §440.41(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''nafcillin sodium capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nafcillin sodium monohydrate used in making the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in §436.204 of this chapter.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59862, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§440.141c Nafcillin sodium monohydrate for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nafcillin sodium monohydrate for oral solution is a packaged combination of one immediate container of nafcillin sodium monohydrate and one immediate container of an aqueous diluent containing one or more suitable and harmless colorings, flavoring, buffers, dispersants, diluents, and preservatives. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 50 milligrams of nafcillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The nafcillin sodium monohydrate used conforms to the standards prescribed by §440.41(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''nafcillin sodium for oral solution''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nafcillin sodium monohydrate used in making the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Place an accurately measured representative aliquot of the sample into a 250-milliliter volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Mix well. Further dilute an aliquot with solution 1 to the reference concentration

of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59862, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.149 Oxacillin sodium monohydrate oral dosage forms.

§ 440.149a Oxacillin sodium monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxacillin sodium monohydrate capsules are composed of oxacillin sodium monohydrate with or without one or more diluents and lubricants, enclosed in a gelatin capsule. Each capsule contains oxacillin sodium monohydrate equivalent to 125, 250, or 500 milligrams of oxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxacillin that it is represented to contain. Its moisture content is not more than 6.0 percent. The oxacillin sodium monohydrate used conforms to the standards prescribed by § 440.49(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''oxacillin sodium capsules''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxacillin sodium monohydrate used in making the batch for potency, moisture, pH, oxacillin content, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59862, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.149b Oxacillin sodium monohydrate for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxacillin sodium monohydrate for oral solution is a mixture of oxacillin sodium monohydrate with one or more suitable colorings, flavorings, buffer substances, stabilizers, and preservatives. When reconstituted as directed in the labeling, each milliliter contains the equivalent of either 25 or 50 milligrams of oxacillin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of

oxacillin that it is represented to contain. Its moisture content is not more than 1.0 percent. When reconstituted as directed in its labeling, the pH of the solution is not less than 5.0 and not more than 7.5. The oxacillin sodium monohydrate used conforms to the standards prescribed by § 440.49(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''oxacillin sodium for oral solution''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxacillin sodium monohydrate used in making the batch for potency, moisture, pH, oxacillin content, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The oxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Place an accurately measured representative aliquot of the sample into an appropriate-sized volumetric flask with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59862, Nov. 22, 1977; 50 FR 19919, May 13, 1985]

§ 440.155 Penicillin G benzathine oral dosage forms.

§§ 440.155a—440.155b [Reserved]

§ 440.155c Penicillin G benzathine oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G benzathine oral suspension contains penicillin G benzathine with one or more suitable dispersing agents, buffer substances, preservatives, colorings, and flavorings. Each milliliter contains penicillin G benzathine equivalent to 30,000 units or 60,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. Its pH is not less than 6.0 and not more than 7.0. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a(a)(1), except sterility and pyrogens.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this

chapter, preparing the sample for assay as follows: Dissolve an accurately measured representative volume of the sample in sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, using a representative aliquot of the drug prepared for assay as described in paragraph (b)(2) of that section.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[42 FR 59862, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.155d Penicillin G benzathine tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G benzathine tablets contain penicillin G benzathine with one or more suitable and harmless diluents, binders, lubricants, colorings, and flavorings. Each tablet contains penicillin G benzathine equivalent to 200,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. Its moisture content is not more than 8.0 percent. The tablets shall disintegrate within 1 hour. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a(a)(1), except sterility and pyrogens.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Using the penicillin G working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing 200 milliliters of absolute methyl alcohol. Blend for 1 minute. Add an additional 300 milliliters of absolute methyl alcohol and blend again for 2 to 3 minutes. Immediately further dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Weigh and finely powder six tablets. Transfer two accurately weighed portions of the tablets, each equivalent to 200,000 units of penicillin G, to two separate 100-milliliter volumetric flasks. Dilute one flask, which is to be used as the blank, to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and proceed as directed in § 436.204(d) of this chapter. In lieu of directions in § 436.204(e) (1), (2), and (3), to the other flask add 10 milliliters of 1.0*N* NaOH and mix well. Allow to stand for 15 minutes, then add 10 milliliters of 1.2*N* HC1, and dilute to volume with distilled water. Pipette a 2.0-milliliter aliquot into a 125-milliliter glass-stoppered Erlenmeyer flask and proceed as directed in § 436.204(c)(4) of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[42 FR 59862, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.171 Penicillin V oral dosage forms.

§ 440.171a Penicillin V capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V capsules are composed of penicillin V with one or more suitable and harmless lubricants. Each capsule contains either 125 milligrams (200,000 units) or 250 milligrams (400,000 units) of penicillin V. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams or units of penicillin V that it is represented to contain. Its moisture content is not more than 2 percent. The penicillin V used conforms to the standards prescribed by § 440.71(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin V used in making the batch for potency, moisture, pH, penicillin V content, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The penicillin V used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient absolute methyl alcohol to give a solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(a) Microbiological agar diffusion assay. Proceed as directed in § 436.105 of this chapter. Immediately dilute an aliquot of the methyl alcohol solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(b) Iodometric assay. Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the methyl alcohol with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 59863, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.171b Penicillin V for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V for oral suspension is composed of penicillin V with or without one or more suitable and harmless suspending agents, colorings, flavorings, and buffer substances. When reconstituted as directed in the labeling, each millilter contains 25 milligrams (40,000 units), 50 milligrams (80,000 units) or 208.3 milligrams (333,333 units) of penicillin V. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams or units of penicillin V that it is represented to contain. Its moisture content is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 4.0. The penicillin V used conforms to the standards prescribed by § 440.71(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The penicillin V used in making the batch for potency, moisture, pH, penicillin V content, and crystallinity.

(b) The batch for potency, moisture, and pH.

(ii) Samples required:

(a) The penicillin V used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dissolve an accurately measured representative volume of the sample in sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately further dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin V (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Dissolve an accurately measured representative portion of the sample in absolute methyl alcohol and dilute with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1) to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample reconstituted as directed in the labeling.

[42 FR 59863, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.171c Penicillin V tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V tablets are composed of penicillin V with or without one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains 125 milligrams (200,000 units), 300 milligrams (500,000 units), or 500 milligrams (800,000 units) of penicillin V. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams or units of penicillin V that it is represented to contain. Its moisture content is not more than 3 percent. It shall disintegrate within 1 hour. The penicillin V used conforms to the standards prescribed by § 440.71(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The penicillin V used in making the batch for potency, moisture, pH, penicillin V content, and crystallinity.

(b) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(a) The penicillin V used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient absolute methyl alcohol to give a stock solution of convenient concentration. Blend for 2 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(a) Microbiological agar diffusion assay. Proceed as directed in § 436.105 of this chapter. Immediately dilute an aliquot of the methyl alcohol solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(b) Iodometric assay. Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the methyl alcohol solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[42 FR 59864, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.173 Penicillin V potassium oral dosage forms.

§ 440.173a Penicillin V potassium capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V potassium capsules are composed of penicillin V potassium and one or more suitable lubricants and fillers. Each capsule contains penicillin V potassium equivalent to 250 milligrams (400,000 units) or 500 milligrams (800,000 units) of penicillin V. The potency is satisfactory if it is not less than 90 percent and more than 115

percent of the number of milligrams or units of penicillin V that it is represented to contain. Its loss on drying is not more than 2.0 percent. The penicillin V potassium used conforms to the standards prescribed by §440.73(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin V potassium used in making the batch for potency, loss on drying, pH, crystallinity, penicillin V content.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The penicillin V potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Using the penicillin V working standard as the standard of comparison, assay by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in §436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[42 FR 59864, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§440.173b Penicillin V potassium chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V potassium chewable tablets are composed of penicillin V potassium with suitable diluents, binders, buffers, colorings, and flavorings. Each tablet contains penicillin V potassium equivalent to 125 milligrams (200,000 units) or 250 milligrams (400,000 units) of penicillin V. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams or units of penicillin V that it is represented to contain. The loss on drying is not more than 1.5 percent. The penicillin V potassium used conforms to the standards prescribed by §440.73(a) (1).

(2) *Labeling.* In addition to the labeling requirements prescribed by §432.5 of this chapter, this drug shall be labeled ''penicillin V potassium tablets''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin V potassium used in making the batch for potency, loss on drying, pH, penicillin V content, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The penicillin V potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the

reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[42 FR 59864, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.173c Penicillin V potassium tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V potassium tablets are composed of penicillin V potassium with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains penicillin V potassium equivalent to 125 milligrams (200,000 units), 250 milligrams (400,000 units), or 500 milligrams (800,000 units) of penicillin V. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams or units of penicillin V that it is represented to contain. Its loss on drying is not more than 1.5 percent. It shall disintegrate within 1 hour. The penicillin V potassium used conforms to the standards prescribed by § 440.73(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin V potassium used in making the batch for potency, loss on drying, pH, penicillin V content and crystallinity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The penicillin V potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescibed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[42 FR 59865, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.173d Penicillin V potassium for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin V potassium for oral solution is composed of penicillin V potassium with or without one or more suitable and harmless suspending agents, colorings, flavorings, buffer substances, and preservatives. When reconstituted as directed in the labeling, each milliliter contains penicillin V potassium equivalent to either 25 milligrams (40,000 units) or 50 milligrams (80,000 units) of penicillin V. Its potency is satisfactory if it contains not less than 90 percent and not more than 135 percent of the number of milligrams or units of penicillin V that it is represented to contain. Its moisture content is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The penicillin V potassium used conforms to the standards prescribed by § 440.73(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such requests shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin V potassium used in making the batch for potency, loss on drying, pH, penicillin V content, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The penicillin V potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Dilute an accurately measured representative portion of the suspension with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin V per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in §436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the sample when reconstituted as directed in the labeling.

[42 FR 59865, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§440.180 Penicillin G potassium oral dosage forms.

§440.180a Penicillin G potassium tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin potassium tablets are composed of penicillin G potassium with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains penicillin G potassium equivalent to 100,000 units, 200,000 units, 250,000 units, 400,000 units, 500,000 units, 800,000 units or 1,000,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. Its loss on drying is not more than 1 percent. The tablets shall disintegrate within 1 hour. The penicillin G potassium used conforms to the standards prescribed by §440.80a(a)(1), except sterility and pyrogens.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(*b*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: Potency, loss on drying, and disintegration time of tablets collected during the time of tableting the batch; and, unless the tablets are packaged into dispensing-size containers immediately after they are compressed or the manufacturer has submitted to the Commissioner, and it has been accepted, information adequate to prove that such tests are not necessary, loss on drying of the tablets collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Potency, loss on drying, and disintegration time of tablets collected during each day the tablets are being packaged into dispensing-size containers.

(ii) Samples required:

(*a*) The penicillin G potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: A minimum of 36 tablets. If, after tableting, such person packaged the batch into dispensing-size containers: 20 tablets, collected at equal intervals during each day the tablets are being packaged, except that this sample is not required if the tablets are packaged immediately after they are compressed or if the manufacturer has been exempted by the Commissioner from such requirement.

(*2*) If the person who requests certification is not the manufacturer of the batch (for the purposes of certification, a batch shall be that number of tablets filled by such person into dispensing-size containers during each day's packaging operations): A minimum of 36 tablets collected by taking single tablets at such intervals throughout each day of packaging the tablets so that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[42 FR 59865, Nov. 22, 1977; 43 FR 3705, Jan. 27, 1978, as amended at 50 FR 19919, May 13, 1985]

§ 440.180c Penicillin G potassium capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G potassium capsules are composed of penicillin G potassium and a suitable and harmless diluent. Each capsule contains penicillin G potassium equivalent to 250,000 units or 400,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. Its loss on drying is not more than 1.5 percent. The penicillin G potassium used conforms to the standards prescribed by § 440.80a(a)(1), except sterility and pyrogens.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(b) The batch:

(1) If the person who requests certification is the manufacturer of the batch: Potency and loss on drying of capsules collected during the time of encapsulating the batch; and, unless the capsules are packaged into dispensing-size containers immediately after they are encapsulated or the manufacturer has submitted to the Commissioner, and it has been accepted, information adequate to prove that such tests are not necessary, loss on drying of capsules collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Potency and loss on drying of capsules collected during each day the capsules are being packaged into dispensing-size containers.

(ii) Samples required:

(*a*) The penicillin G potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: A minimum of 30 capsules. If after encapsulating, such person packaged the batch into dispensing-size

containers: 20 capsules collected at equal intervals during each day the capsules are being packaged, except that this sample is not required if the capsules are packaged immediately after they are filled or if the manufacturer has been exempted by the Commissioner from such requirement.

(*2*) If the person who requests certification is not the manufacturer of the batch (for the purposes of certification, a batch shall be that number of capsules filed by such person into dispensing-size containers during each day's packaging operations): A minimum of 30 capsules collected by taking single capsules at such intervals throughout each day of packaging the capsules that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation*. Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures*. Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in §436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying*. Proceed as directed in §436.200(b) of this chapter.

[42 FR 59866, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§440.180f Penicillin G potassium for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Penicillin G potassium for oral solution contains penicillin G potassium and one or more suitable and harmless buffers, colorings, flavorings, diluents, and preservatives. Each milliliter contains penicillin G potassium equivalent to 20,000 units, 25,000 units, 40,000 units, 50,000 units, 80,000 units, or 100,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of penicillin G that it is represented to contain. Its moisture content is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The penicillin G potassium used conforms to the standards prescribed by §440.80a(a)(1), except sterility and pyrogens.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The penicillin G potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation*. Reconstitute as directed in the labeling. Transfer an accurately measured representative portion into a suitable volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1).

(ii) *Assay procedures*. Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in §436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in §436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained after reconstituting the drug as directed in the labeling.

[42 FR 59866, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.180g Penicillin G potassium tablets for solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G potassium tablets for solution are composed of penicillin G potassium. Each tablet contains penicillin G potassium equivalent to 100,000 units, 200,000 units, or 250,000 units of penicillin G. The potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. Its loss on drying is not more than 1 percent. The penicillin G potassium used conforms to the standards prescribed by § 440.80a(a)(1), except sterility and pyrogens.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(*b*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: Potency and loss on drying of tablets collected during the time of tableting the batch; and, unless the tablets are packaged into dispensing-size containers immediately after they are compressed, or the manufacturer has submitted to the Commissioner, and it has been accepted, information adequate to prove that such tests are not necessary, loss on drying of the tablets collected during each day of packaging the batch.

(*2*) If the person who requests certification is not the manufacturer of the batch: Potency and loss on drying of the tablets collected during each day the tablets are being packaged into dispensing-size containers.

(ii) Samples required:

(*a*) The penicillin G potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the person who requests certification is the manufacturer of the batch: A minimum of 30 tablets. If, after tableting, such person packaged the batch into dispensing-size containers: 20 tablets collected at equal intervals during each day the tablets are packaged, except that this sample is not required if the tablets are packaged immediately after they are compressed or if the manufacturer has been exempted by the Commissioner from such requirement.

(*2*) If the person who requests certification is not the manufacturer of the batch (for the purposes of certification, a batch shall be that number of tablets filed by such person into dispensing-size containers during each day's packaging operations): A minimum of 30 tablets collected by taking single tablets at such intervals throughout each day of packaging the tablets that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[42 FR 59867, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 440.201 Sterile azlocillin sodium.

The requirements for certification and the tests and methods of assay for sterile azlocillin sodium packaged for dispensing are described in § 440.1a.

[47 FR 53349, Nov. 26, 1982]

§ 440.202 Sterile amdinocillin.

The requirements for certification and the tests and methods of assay for sterile amdinocillin packaged for dispensing are described in § 440.2a.

[50 FR 7766, Feb. 26, 1985]

§ 440.207 Sterile ampicillin trihydrate for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile ampicillin trihydrate for suspension is a dry mixture of ampicillin trihydrate and one or more suitable and harmless buffer substances, stabilizers, suspending agents, and preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not less than 11.4 percent and not more than 14.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.0. The ampicillin trihydrate used conforms to the standards prescribed by § 440.7a(a)(1) of this chapter.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''sterile ampicillin for suspension.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ampicillin trihydrate used in making the batch for potency, loss on drying, pH, ampicillin content, concordance, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The ampicillin trihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the resultant solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), for the microbiological agar diffusion assay, or distilled water for the iodometric assay, to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, except in paragraph (d) of that section, add 3 drops of 1.2*N* hydrochloric acid to both the sample and working standard solutions after the addition of 0.01*N* iodine solution. Dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except in lieu of (e)(1)(i)(*a*), prepare the sample for test as follows: From each of 10 immediate containers,

aseptically transfer approximately 300 milligrams of sample into a sterile 500-milliliter Erlenmeyer flask containing approximately 400 milliliters of diluting fluid D. Add at least 200,000 Levy units[1] of penicillinase. Repeat the process using 10 additional containers. Swirl both of the stoppered flasks to completely solubilize the suspension prior to filtration and proceed as directed in paragraph (e)(1)(ii) of that section. If the formulation cannot be filtered, proceed as directed in § 436.20(e)(2) of this chapter, except use medium B in lieu of medium A.

(3) *Pyrogens.* Proceed as directed in § 436.32(f) of this chapter, using a solution containing 20 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 49 FR 3459, Jan. 27, 1984; 50 FR 19918, 19919, May 13, 1985]

§ 440.209 Ampicillin sodium injectable dosage forms.

§ 440.209a Sterile ampicillin sodium.

The requirements for certification and the tests and methods of assay for sterile ampicillin sodium packaged for dispensing are described in § 440.9a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977. Redesignated at 52 FR 42288, Nov. 4, 1987]

§ 440.209b Sterile ampicillin sodium and sulbactam sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ampicillin sodium and sulbactam sodium is a dry mixture of ampicillin sodium and sulbactam sodium in which the ratio of ampicillin to sulbactam is 2:1. Its ampicillin potency is not less than 563 micrograms of ampicillin per milligram on an anhydrous basis. It contains not less than 280 micrograms of sulbactam per milligram on an anhydrous basis. Its ampicillin sodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ampicillin that it is represented to contain. Its sulbactam sodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of sulbactam that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. The pH of an aqueous solution containing 10 milligrams of ampicillin and 5 milligrams of sulbactam per milliliter is not less than 8.0 and not more than 10.0. It passes the identity test for ampicillin and sulbactam. The ampicillin sodium content conforms to the standards prescribed by § 440.9a(a)(1) of this chapter. The sulbactam content conforms to the standards prescribed by § 455.82a(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The ampicillin sodium used in making the batch for potency, sterility, pyrogens, moisture, pH, crystallinity, and identity.

(B) The sulbactam sodium used in making the batch for potency, sterility, pyrogens, moisture, crystallinity, and identity.

(C) The batch for ampicillin potency, sulbactam potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The ampicillin sodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(B) The sulbactam sodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(C) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

[1] One Levy unit of penicillinase inactivates 59.3 units of pencillin G in 1 hour at 25° C. and at a pH of 7.0 in a phosphate buffered solution of a pure alkali salt of penicillin G when the substrate is in sufficient concentration to maintain a zero order reaction.

(*2*) For sterility testing: A minimum of 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ampicillin and sulbactam content.* Proceed as directed in § 436.216 of this chapter, operating isothermally at 25 °C, using an ultraviolet detection system operating at a wavelength of 230 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0M Phosphoric acid.* Prepare by diluting 67.5 milliliters of reagent grade phosphoric acid (85 percent) in distilled water to 1 liter.

(B) *0.005M Tetrabutylammonium hydroxide.* Dilute 6.6 milliliters of tetrabutylammonium hydroxide (40 percent) to 1,800 milliliters with distilled water. Adjust the pH to 5.0 with 1.0*M* phosphoric acid and dilute with distill water to 2 liters.

(C) *Mobile phase.* Mix 350 milliliters of acetonitrile with 1,650 milliliters of 0.005*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chromatograph pumping system. (Slight adjustments in pH and/or acetonitrile content may be made to achieve the system suitability parameters defined in paragraph (b)(1)(iii) of this section.)

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Accurately weigh a portion of the ampicillin working standard containing the equivalence of approximately 75 milligrams of ampicillin activity and transfer into a 25-milliliter volumetric flask. Accurately weigh a portion of the sulbactam working standard containing 35 milligrams of sulbactam and transfer into the 25-milliliter volumetric flask containing the ampicillin. Dissolve and dilute to volume with mobile phase. Further dilute 5 milliliters to 25 milliliters with mobile phase.

(B) *Sample solution.* Dissolve an accurately weighed sample in sufficient mobile phase to give a stock solution containing 1 milligram of sample per milliliter (estimated); and, also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with mobile phase to yield a solution containing about 0.30 milligram sulbactam and about 0.60 milligram ampicillin per milliliter.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 3,500 theoretical plates for sulbactam for a 30-centimeter column.

(C) *Resolution.* Dissolve 17.5 milligrams of sulbactam in 50 milliliters of 0.01*N* sodium hydroxide and let stand for 30 minutes. Adjust the pH of the solution to 5.0 with concentrated phosphoric acid. Transfer a 5-milliliter aliquot of the resulting solution to a 25-milliliter volumetric flask, add 4.25 milliliters of acetonitrile, and dilute to volume with 0.005*M* tetrabutylammonium hydroxide as described in paragraph (b)(1)(i)(B) of this section. Transfer 2 milliliters of this solution to a 50-milliliter flask, add 30 milligrams of ampicillin potency, dissolve and dilute to volume with mobile phase. Use this solution to determine the resolution factor. The resolution (*R*) between the peaks for ampicillin and sulbactam alkaline degradation product is satisfactory if it is not less than 1.2.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations.* (A) Calculate the micrograms of ampicillin or sulbactam per milligram of sample as follows:

$$\text{Micrograms of ampicillin or sulbactam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the ampicillin or sulbactam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ampicillin or sulbactam peak in the chromatogram of the ampicillin or sulbactam working standard;

P_s=Ampicillin or sulbactam activity in the ampicillin-sulbactam working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(B) Calculate the ampicillin or sulbactam content of the container as follows:

$$\text{Milligrams of ampicillin or sulbactam per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ampicillin or sulbactam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ampicillin or sulbactam peak in the chromatogram of the ampicillin or sulbactam working standard;

P_s=Ampicillin or sulbactam activity in the ampicillin-sulbactam working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20 milligrams of sulbactam and 40 milligrams of ampicillin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of ampicillin and 5 milligrams of sulbactam per milliliter.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ampicillin-sulbactam working standard.

[52 FR 42288, Nov. 4, 1987, as amended at 54 FR 47205, Feb. 20, 1989; 55 FR 11582, Mar. 29, 1990]

§ 440.210 Benzylpenicilloyl-polylysine injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Benzylpenicilloyl-polylysine injection is an aqueous solution of benzylpenicilloyl-polylysine. It contains one or more suitable and harmless buffers. Its benzylpenicilloyl content is satisfactory if it is not less than $5.4\times10^{-5}M$ and not more than $7.0\times10^{-5}M$, except that for the issuance of a certificate for a batch, the benzylpenicilloyl content must be not less than $6.4\times10^{-5}M$. It is sterile. It is nonpyrogenic. Its pH is not less than 6.5 and not more than 8.5. The benzylpenicilloyl-polylysine concentrate used conforms to the standards prescribed by § 440.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The benzylpenicilloyl-polylysine concentrate used in making the batch for percent benzylpenicilloyl substitution, benzylpenicilloyl content, penamaldate content, penicillenate content, and pH.

(*b*) The batch for benzylpenicilloyl content, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The benzylpenicilloyl-polylysine concentrate used in making the batch: 2 vials, each containing not less than 5 milliliters.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 60 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Benzylpenicilloyl content.* Proceed as directed in §440.10(b)(1)(ii) except in lieu of §440.10(b)(1)(ii)(*b*) prepare the sample solution as follows: Pool contents of 16 immediate containers. Dilute a 3.0-milliliter aliquot to 10 milliliters with saline phosphate buffer, pH 7.6.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, preparing the sample solution as follows: Pool the contents of at least 8 vials to obtain a minimum of 1.5 milliliters of the original preparation. Dilute the 1.5 milliliters to 50 milliliters with diluent 2.

(4) [Reserved]

(5) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

[39 FR 35347, Oct. 1, 1974, as amended at 42 FR 14094, Mar. 15, 1977; 50 FR 19918, 19919, May 13, 1985]

§440.213 Sterile carbenicillin disodium.

The requirements for certification and the tests and methods of assay for sterile carbenicillin disodium packaged for dispensing are described in §440.13a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977]

§440.219 Dicloxacillin sodium monohydrate injectable dosage forms.

§440.219a Sterile dicloxacillin sodium monohydrate.

The requirements for certification and the tests and methods of assay for sterile dicloxacillin sodium monohydrate packaged for dispensing are described in §440.19a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977]

§440.219b Dicloxacillin sodium monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dicloxacillin sodium monohydrate for injection is a dry mixture of dicloxacillin sodium monohydrate and lidocaine hydrochloride packaged for dispensing. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of dicloxacillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.5. The dicloxacillin sodium monohydrate used conforms to the standards prescribed by §440.19a(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''dicloxacillin sodium for injection''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The dicloxacillin sodium monohydrate used in making the batch for potency, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The dicloxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay or in distilled water for the iodometric assay and hydroxylamine

colorimetric assay, to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with distilled water to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, except dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of dicloxacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the product reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977; 50 FR 19918, 19919, May 13, 1985]

§ 440.229 Hetacillin potassium injectable dosage forms.

§ 440.229a Sterile hetacillin potassium.

The requirements for certification and the tests and methods of assay for sterile hetacillin potassium packaged for dispensing are described in § 440.29a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977]

§ 440.229b Hetacillin potassium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin potassium for injection is a dry mixture of hetacillin potassium and lidocaine hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain. It is sterile and nonpyrogenic. Its moisture content is not more than 1.0 percent. When reconstituted as directed in its labeling, its pH is not less than 7.0 and not more than 9.0. The hetacillin potassium used conforms to the requirements of § 440.29a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The hetacillin potassium used in making the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The hetacillin potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers, except if each contains less than 450 milligrams of ampicillin, a minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution

3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing the equivalent of 18 milligrams of ampicillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the product reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59867, Nov. 22, 1977; 50 FR 19918, 19919, May 13, 1985]

§ 440.236 Methicillin sodium monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methicillin sodium monohydrate for injection is methicillin sodium monohydrate with or without one or more suitable and harmless preservatives and the buffer sodium citrate in a quantity not less than 4 percent and not more than 5 percent by weight of its total solids (such sodium citrate conforms to the standards prescribed therefor by the U.S.P.). Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of methicillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.0 and not more than 8.5. Its moisture content is not more than 6.0 percent. The methicillin sodium monohydrate used conforms to the standards prescribed by § 440.36a(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''methicillin sodium for injection''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methicillin sodium monohydrate used in making the batch for potency, moisture, pH, methicillin content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, pH, and moisture.

(ii) Samples required:

(*a*) The methicillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams, plus one package containing approximately 2 grams.

(*b*) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the portion thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of methicillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 60 milligrams of methicillin per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per millilter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

[39 FR 18976, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975; 42 FR 59868, Nov. 22, 1977; 49 FR 5096, Feb. 10, 1984; 50 FR 19918, 19919, May 13, 1985]

§ 440.237 Sterile mezlocillin sodium monohydrate.

The requirements for certification and the tests and methods of assay for sterile mezlocillin sodium monohydrate packaged for dispensing are described in § 440.37a.

[46 FR 58299, Dec. 1, 1981]

§ 440.241 Nafcillin sodium injectable dosage forms.

§ 440.241a Nafcillin sodium monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nafcillin sodium monohydrate for injection is a dry mixture of nafcillin sodium monohydrate and a suitable buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not less than 3.5 and not more than 5.3 percent. When reconstituted as directed in the labeling, the pH is not less than 6.0 and not more than 8.5. The nafcillin sodium monohydrate used conforms to the requirements of § 440.41a(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''nafcillin sodium for injection''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nafcillin sodium monohydrate used in making the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation remove an accurately measured representative portion from each container. Dilute the sample thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 2.0 micrograms of nafcillin per milliliter (estimated) for the microbiological agar diffusion assay and to the prescribed concentration for the iodometric assay.

(ii) *Assay procedures.* Assay for potency by either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter.

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 80 milligrams of nafcillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59868, Nov. 22, 1977; 45 FR 22922, Apr. 4, 1980; 47 FR 22515, May 25, 1982; 50 FR 19919, May 13, 1985. Redesignated at 55 FR 277, Jan. 4, 1990]

§440.241b Nafcillin sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nafcillin sodium injection is a frozen, aqueous, iso-osmatic solution of nafcillin sodium which may contain one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains nafcillin sodium equivalent to 20 or 40 milligrams of nafcillin. Its nafcillin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of nafcillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 6.0 and not more than 8.5. The nafcillin sodium monohydrate used conforms to the standards prescribed by §440.41(a)(1).

(2) *Labeling.* it shall be labeled in accordance with the requirements of §432.5 of this chapter. In addition, this drug shall be labeled ''nafcillin sodium injection.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The nafcillin sodium monohydrate used in making the batch for potency, moisture, pH, crystallinity, nafcillin content, and identity.

(B) The batch for nafcillin content, sterility, pyrogens, and pH.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The nafcillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Nafcillin content.* Proceed as directed in §440.241a(b)(1), except use the thawed solution.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 80 milligrams of nafcillin per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

[55 FR 277, Jan. 4, 1990]

§440.249 Oxacillin sodium injectable dosage forms.

§440.249a Oxacillin sodium monohydrate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxacillin sodium monohydrate for injection is a dry mixture of oxacillin sodium monohydrate and one or more buffer substances, with or without trisodium ethylenediamine tetraacetic acid, and with or without one or more suitable and harmless preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxacillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 6.0 percent. Its pH in an aqueous solution containing 30 milligrams per milliliter is not less than 6.0 and not more than 8.5. The oxacillin sodium monohydrate used conforms to the standards prescribed by §440.49a(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''oxacillin sodium for injection''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxacillin sodium monohydrate used in making the batch

for potency, moisture, pH, oxacillin content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The oxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, or 40 immediate containers if each contains less than 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of oxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of oxacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59868, Nov. 22, 1977; 50 FR 19918, 19919, May 13, 1985. Redesignated at 55 FR 279, Jan. 4, 1990]

§ 440.249b Oxacillin sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxacillin sodium injection is a frozen aqueous, iso-osmotic solution of oxacillin sodium which may contain one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains oxacillin sodium equivalent to 20 or 40 milligrams of oxacillin. Its oxacillin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxacillin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 6.0 and not more than 8.5. The oxacillin sodium monohydrate used conforms to the standards prescribed by § 440.49(a)(1), except that the pH of an aqueous solution containing 30 milligrams per milliliter is not less than 4.0 and not more than 7.0.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, this drug shall be labeled ''oxacillin sodium injection''.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The oxacillin sodium monohydrate used in making the batch for potency, moisture, pH, oxacillin content, crystallinity, and identity.

(B) The batch for oxacillin content, sterility, pyrogens, and pH.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The oxacillin sodium monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. he sample solution used for testing must be at room temperature.

(1) *Oxacillin content.* Proceed as directed in §440.249a(b)(1), except use the thawed solution.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 20 milligrams of oxacillin per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

[55 FR 279, Jan. 4, 1990; 55 FR 2481, Jan. 24, 1990]

§440.255 Penicillin G benzathine injectable dosage forms.

§440.255b Sterile penicillin G benzathine suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile penicillin G benzathine suspension is an aqueous suspension of penicillin G benzathine and one or more suitable suspending or dispersing agents, buffer substances, and preservatives. Each container or each milliliter contains penicillin G benzathine equivalent to not less than 300,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. The penicillin G benzathine used conforms to the standards prescribed by §440.55a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filing operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume, remove an accurately measured representative portion from each container. Dilute the portion thus obtained with sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume, remove an accurately measured representative portion from each container. Using the sample thus obtained, proceed as directed in §436.204(b)(2) of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of

medium E. During the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[42 FR 59868, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 50 FR 19918, 19919, May 13, 1985]

§ 440.255c Sterile penicillin G benzathine-penicillin G procaine suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile penicillin G benzathine-penicillin G procaine suspension is an aqueous mixture of penicillin G benzathine and penicillin G procaine with or without suitable and harmless buffer substances, suspending agents, and preservatives. Each container or each milliliter contains penicillin G benzathine and penicillin G procaine each equivalent to not less than 150,000 units of penicillin G. Its penicillin G benzathine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. Its penicillin G procaine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a (a)(1). The penicillin G procaine used conforms to the standards prescribed by § 440.74a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The penicillin G procaine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*c*) The batch for penicillin G benzathine content, penicillin G procaine content, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The penicillin G procaine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regualr intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Total potency.* Assay for total potency by either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, place one dose of the drug in a 100-milliliter volumetric flask and add sufficient methyl alcohol to dissolve the benzathine penicillin G. Dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Immediately further dilute an aliquot with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Using a suitable hypodermic needle and syringe, withdraw 2 one-dose portions of sample. Place one portion into an appropriate-sized volumetric flask and add 20 milliliters of 0.5*N* NaOH for each 300,000 units of benzathine penicillin G, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2*N* HCl for each 2 milliliters of 0.5*N* NaOH,

mix, and dilute with distilled water to obtain a concentration of 2,000 units per milliliter. Dilute the other portion, which is to be used as the blank solution, with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a concentration of approximately 2,000 units per milliliter.

(ii) *Penicillin G procaine content*—(*a*) *Reagents*—(*1*) *Sodium nitrite solution*. Dissolve 0.1 gram of sodium nitrite in 100 milliliters of distilled water. Prepare a fresh solution every week and store under refrigeration.

(*2*) *Ammonium sulfamate solution*. Dissolve 0.5 gram of ammonium sulfamate in 100 milliliters of distilled water and store under refrigeration.

(*3*) *N-(1-naphthyl)-ethylenediamine solution*. Dissolve 0.1 gram of *N*-(1-naphthyl) - ethylenediamine dihydrochloride in 100 milliliters of distilled water. Prepare fresh solutions every week and store under refrigeration.

(4) Standard procaine solution. Prepare a standard solution containing 27.55 milligrams of procaine hydrochloride U.S.P. in a liter of distilled water (each milliliter of the standard solution is equivalent to 60 units of penicillin G procaine).

(*b*) *Preparation of sample solution*. Using a suitable hypodermic needle and syringe, withdraw a one-dose portion of the sample and place it into an appropriate-sized volumetric flask. Add 20 milliliters of 0.5*N* NaOH for each 300,000 units of penicillin G benzathine, mix well, being sure that all penicillin is in solution, and allow to stand for 15 minutes. Add 1 milliliter of 1.2*N* HC1 for each 2 milliliters of 0.5*N* NaOH, mix, and dilute with distilled water to obtain a concentration of 60 units of penicillin G procaine per milliliter. Transfer a 3.0-milliliter aliquot of this solution to a 50-milliliter volumetric flask and add 2 milliliters of water to give a volume of 5 milliliters.

(*c*) *Procedure*. Transfer respectively, 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of the standard procaine solution to each of five 50-milliliter volumetric flasks and transfer 5.0 milliliters of distilled water to a sixth 50-milliliter volumetric flask. Add 4.0, 3.0, 2.0, and 1.0 milliliters of water to the first four flasks, respectively, to give each a volume of 5 milliliters. To each flask of the standard and sample solutions, add 0.5 milliliter of 4*N* HC1, 1.0 milliliter of sodium nitrite solution, 1.0 milliliter of ammonium sulfamate solution, and 1.0 milliliter of *N*-(1-naphthyl)-ethylenediamine solution. Mix and wait two minutes after each addition. Dilute each flask to volume with distilled water. Using a suitable photoelectric colorimeter, determine the absorbancy of each solution at 550 nanometers. The instrument is balanced so that the zero concentration reads 0 absorbancy. Plot the standard curve on coordinate graph paper. Obtain the procaine penicillin content of the solution for assay directly from the point on the standard curve corresponding to its absorbancy.

(iii) *Penicillin G benzathine content*. The sum of the penicillin G procaine content determined as directed in paragraph (b)(1)(ii) of this section subtracted from the total potency determined as directed in paragraph (b)(1)(i) of this section represents the penicillin G benzathine content.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, medium F in lieu of medium E, and during the period of incubation, shake the tubes at least once daily.

(3) *Pyrogens*. Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted aqueous suspension.

[42 FR 59868, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 50 FR 19918, 19919, May 13, 1985]

§ 440.255d Sterile penicillin G benzathine for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile penicillin G benzathine for suspension is a dry mixture of penicillin G benzathine and one or more suitable suspending or dispersing agents, and with or without one or more suitable preservatives and buffer substances. Its potency is satisfactory if it is not less than 90 percent and not

more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not less than 5.0 percent and not more than 8.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The penicillin G benzathine used conforms to the standards prescribed by § 440.55a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G benzathine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The penicillin G benzathine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the batch is packaged for repacking:

(*i*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*ii*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(*2*) If the batch is packaged for dispensing:

(*i*) For all tests except sterility: A minimum of 10 immediate containers.

(*ii*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single–dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dissolve the portion thus obtained with sufficient absolute methyl alcohol to give a solution of convenient concentration. Immediately, further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Using the sample thus obtained, proceed as directed in § 436.205(b)(2) of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium C in lieu of medium A, and medium F in lieu of medium E. During the period of incubation shake the tubes at least once daily.

(3) *Pyrogens.* Proceed as directed in § 436.32(d) of this chapter, using a solution containing 4,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension obtained when the product is reconstituted as directed in the labeling.

[42 FR 59869, Nov. 22, 1977, as amended at 50 FR 19918, 19919, May 13, 1985]

§ 440.274 Penicillin G procaine injectable dosage forms.

§ 440.274a Sterile penicillin G procaine with aluminum stearate suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Sterile penicillin G procaine with aluminum stearate suspension is penicillin G procaine in a refined vegetable oil with one or more suitable and harmless dispersing agents and hardening agents. Each milliliter contains penicillin G procaine equivalent to 300,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. Its moisture content is not more than 1.4 percent. The penicillin G procaine used conforms to the standards prescribed by § 440.74a(a)(1). If the hardening agent is a refined hydrogenated and deodorized peanut oil, it is free from rancidity; it has an iodine value of not more than 10; its free fatty acid content as oleic acid is not more than one-tenth of 1 percent and its melting point is 64° C±2° C.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G procaine used in making the batch for potency, pyrogens, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The penicillin G procaine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample or the entire contents of a single-dose container into a 50-milliliter volumetric flask. Add 4 milliliters of chloroform and mix thoroughly. Dilute to volume with absolute ethyl alcohol. Mix well. Immediately further dilute with sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1 unit of penicillin G per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 59870, Nov. 22, 1977, as amended at 50 FR 19919, May 13, 1985]

§ 440.274b Sterile penicillin G procaine suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile penicillin G procaine suspension is an aqueous mixture of penicillin G procaine and one or more suitable suspending or dispersing agents, buffer substances, and preservatives. It may contain procaine hydrochloride in a concentration not exceeding 2.0 percent and one or more suitable stabilizing agents. Each container or each milliliter contains penicillin G procaine equivalent to not less than 300,000 units of penicillin G. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. The penicillin G procaine used conforms to the standards prescribed by § 440.74a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G procaine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The penicillin G procaine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents, if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume, remove an accurately measured representative portion from each container. Dissolve the sample thus obtained in 50 to 100 milliliters of absolute methyl alcohol and add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Immediately further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents, if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume, remove an accurately measured representative portion from each container. Dissolve and dilute the sample thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed concentration.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase to diluting fluid A and swirl the flask to completely solubilize the procaine penicillin before filtration. If the product contains lecithin, use diluting fluid D in lieu of diluting fluid A. If the product contains sodium carboxymethylcellulose, add sufficient sterile carboxymethylcellulase to diluting fluid A or D to completely solubilize the sodium carboxymethylcellulose before filtration. If the preparation contains homogenizers or suspending agents that prevent solubilization, proceed as directed in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Pyrogens*. Proceed as directed in § 436.32(h) of this chapter, using a solution containing 2,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[42 FR 59870, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.274c Sterile penicillin G procaine for suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile penicillin G procaine for suspension is a dry mixture of penicillin G procaine and one or more suitable suspending or dispersing agents, buffer substances, and preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not less than 2.8 and not more than 4.2 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The penicillin G procaine used conforms to the standards prescribed by § 440.74a(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G procaine used in making the batch for potency, moisture, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The penicillin G procaine used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) If the batch is packaged for repacking:

(*i*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*ii*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(*2*) If the batch is packaged for dispensing:

(*i*) For all tests except sterility: A minimum of 10 immediate containers.

(*ii*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: If it is packaged for repacking, dissolve an accurately weighed sample, equivalent to one dose, in 50 to 100 milliliters of absolute methyl alcohol and add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents, if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dissolve the sample thus obtained in 50 to 100 milliliters of absolute methyl alcohol and add sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Immediately further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: If it is packaged for repacking, dissolve and dilute an accurately weighed sample, equivalent to one dose, with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents, if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume, remove an accurately measured representative portion from each container. Dissolve and dilute the sample thus obtained with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed concentration.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add sufficient penicillinase to diluting fluid A and swirl the flask to completely solubilize the procaine penicillin before filtration. If the product contains lecithin, use diluting fluid D in lieu of diluting fluid A. If the product contains sodium carboxymethylcellulose, add sufficient sterile carboxymethylcellulase to diluting fluid A or D to completely solubilize the sodium carboxymethylcellulose before filtration. If the preparation contains homogenizers or suspending agents that prevent solubilization, proceed as directed in paragraph (e)(2) of that section, except use medium B in lieu of medium A.

(3) *Pyrogens*. Proceed as directed in § 436.32(h) of this chapter, using a solution containing 2,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the suspension obtained when reconstituted as directed in the labeling.

[42 FR 59871, Nov. 22, 1977, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.280 Penicillin G potassium injectable dosage forms.

§ 440.280a Sterile penicillin G potassium.

The requirements for certification and the tests and methods of assay for sterile penicillin G potassium packaged for dispensing are described in § 440.80a.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59871, Nov. 22, 1977]

§ 440.280b Penicillin G potassium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G potassium for injection is a dry mixture of penicillin G potassium and the buffer sodium citrate in a quantity not less than 4.0 percent and not more than 5.0 percent by weight of its total solids. It may contain citric acid in a quantity not more than 0.15 percent of its total solids in place of a corresponding amount of sodium citrate. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 1.5 percent. Its pH is not less than 6.0 and not more than 8.5. If penicillin G potassium buffered is used, it conforms to the standards prescribed by § 440.1080(a)(1). If penicillin G potassium is used, it conforms to the standards prescribed by § 440.80a(a)(1) and the sodium citrate and citric acid conforms to the standards prescribed by the U.S.P.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The penicillin G potassium, buffered, used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove or expel an accurately measured representative portion from each container. Dilute with solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter or, if the diluent is included in a disposable syringe combination, use the solution obtained when the drug is

reconstituted as directed in the labeling.

[42 FR 59871, Nov. 22, 1977, as amended at 43 FR 9799, Mar. 10, 1978; 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§440.280c Penicillin G potassium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G potassium injection is a frozen, aqueous, iso-osmotic solution of penicillin G potassium which may contain one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains penicillin G potassium equivalent to 20,000, 40,000, or 60,000 units of penicillin G. Its penicillin G content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.5 and not more than 8.0. The penicillin G potassium used conforms to the standards prescribed by §440.80(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter. In addition, this drug shall be labeled ''penicillin G potassium injection''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The penicillin G potassium used in making the batch for potency, loss on drying, pH, penicillin G content, and crystallinity.

(B) The batch for penicillin G content, sterility, pyrogens, and pH.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The penicillin G potassium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Penicillin G content.* Proceed as directed in §440.280b(b)(1) of this chapter, except use the thawed solution.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 20,000 units of penicillin G per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

[55 FR 38675, Sept. 20, 1990]

§440.281 Pencillin G sodium injectable dosage forms.

§440.281a Sterile penicillin G sodium.

The requirements for certification and the tests and methods of assay for sterile penicillin G sodium packaged for dispensing are described in §440.81a.

[42 FR 59872, Nov. 22, 1977]

§440.281b Penicillin G sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G sodium for injection is a dry mixture of penicillin G sodium and the buffer sodium citrate in a quantity not less than 4.0 percent and not more than 5.0 percent by weight of its total solids. It may contain citric acid in a quantity not more than 0.15 percent of its total solids in place of a corresponding amount of sodium citrate. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of penicillin G that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 1.5 percent. Its pH is not less than 6.0 and not more than 7.5. The penicillin G sodium, buffered, used conforms to the standards prescribed by §440.1081a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G sodium, buffered, used in making the batch for potency, loss on drying, pH, penicillin G content, crystallinity, and heat stability.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The penicillin G sodium, buffered, used in making the batch: 10 packages, each containing approximately 60 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

[42 FR 59872, Nov. 22, 1977; 43 FR 2393, Jan. 17, 1978, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.283 Sterile piperacillin sodium.

The requirements for certification and the tests and methods of assay for sterile piperacillin sodium packaged for dispensing are described in § 440.83a

[47 FR 15770, Apr. 13, 1982]

§ 440.290 Ticarcillin disodium injectable dosage forms.

§ 440.290a Sterile ticarcillin disodium.

The requirements for certification and the tests and methods of assay for sterile ticarcillin disodium packaged for dispensing are described in § 440.90a.

[43 FR 9800, Mar. 10, 1978. Redesignated at 50 FR 33518, Aug. 20, 1985]

§ 440.290b Sterile ticarcillin disodium and clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ticarcillin disodium and clavulanate potassium is a dry mixture of ticarcillin disodium and clavulanate potassium, in which the ratio of ticarcillin to clavulanic acid is 15:1 or 30:1. Its ticarcillin potency is not less than 755 micrograms of ticarcillin per milligram on an anhydrous basis if the ratio is 30:1 and 733 micrograms of ticarcillin per milligram on an anhydrous basis if the ratio is 15:1. Its ticarcillin disodium content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ticarcillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of

clavulanic acid that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 4.2 percent. Its pH of an aqueous solution containing 100 milligrams per milliliter is not less than 5.5 and not more than 7.5. The ticarcillin disodium conforms to the standards prescribed by § 440.90a(a)(1) except that it contains not less than 840 micrograms of ticarcillin per milligram on an anhydrous basis. The clavulanate potassium conforms to the standards prescribed by § 455.15a(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ticarcillin disodium used in making the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(*b*) The clavulanate potassium used in making the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(*c*) The batch for ticarcillin potency, ticarcillin content, clavulanic acid content, sterility, pyrogens, moisture, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The ticarcillin disodium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*b*) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: A minimum of 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ticarcillin and clavulanic acid contents.* Determine micrograms of ticarcillin per milligram of sample and milligrams of both ticarcillin and clavulanic acid per container. Proceed as directed in § 436.355 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silicas. Reagents, working standard and sample solutions, system suitability requirements, and calculations for ticarcillin or clavulanic acid content are as follows:

(i) *Reagents*—(*a*) *0.1M Monobasic sodium phosphate buffer solution, pH 4.3.* Transfer 13.8 grams of monobasic sodium phosphate monohydrate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.3±0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix acetonitrile: 0.1*M* monobasic sodium phosphate buffer solution, pH 4.3 (5:95 v/v) and mix for no less than two minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with the helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of acetonitrile to aqueous buffer as necessary to obtain satisfactory separation of the peaks.

(*c*) *Diluent.* 0.05*M* monobasic sodium phosphate buffer solution, pH 6.4 Transfer 6.9 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of water. Adjust the pH to 6.4 with sodium hydroxide (10*N*). Dilute to volume with distilled water. Mix well. Use this diluent to prepare the working standard and sample solutions described in paragraph (b)(1)(ii) of this section.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Accurately weigh a quantity of the ticarcillin working standard containing the equivalent of approximately 90 milligrams of ticarcillin activity and transfer into a 100-milliliter volumetric flask. Prepare a solution of the clavulanic acid working standard containing the equivalent of 30 milligrams or 60 milligrams of clavulanic acid activity in a 100-milliliter volumetric flask. Dissolve and dilute to

volume with diluent. Transfer 10 milliliters of this solution into the flask containing the ticarcillin standard. Dilute the combined standard solution to volume with diluent. Mix. Use within 8 hours after preparation.

(*b*) *Preparation of sample solutions*—(*1*) *Ticarcillin potency (micrograms of ticarcillin per milligram).* Accurately weigh the total contents of a container and dissolve with sufficient diluent to obtain a stock solution containing approximately 30 milligrams of ticarcillin per milliliter. Further dilute this solution with diluent to obtain a final concentration of 0.9 milligrams of ticarcillin per milliliter (estimated).

(*2*) *Ticarcilin and clavulanic acid content (milligrams of ticarcillin and clavulanic acid per container).* Reconstitute the container with an appropriate volume of distilled water. Using a suitable hypodermic syringe, remove all of the withdrawable contents. Dilute with diluent to obtain a stock solution containing approximately 30 milligrams of ticarcillin per milliliter and 1 or 2 milligrams of clavulanic acid per milliliter. Further dilute this solution with the diluent to obtain a final concentration of 0.9 milligram of ticarcillin per milliliter. The final solution will contain either 0.03 or 0.06 milligram of clavulanic acid per milliliter (estimated) depending on the initial ticarcillin to clavulanic acid ratio.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2.0.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates in a 25-centimeter column.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and ticarcillin peaks is satisfactory if it is not less than 5.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.355(b) of this chapter.

(iv) *Calculations.* (*a*) Calculate the micrograms of ticarcillin per milligram as follows:

$$\text{Micrograms of ticarcillin per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the ticarcillin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ticarcillin peak in the chromatogram of the ticarcillin working standard;

P_s=Ticarcillin activity in the ticarcillin working standard solution in micrograms of anhydrous ticarcillin free acid per milliliter; and

C_u=Milligrams of sample per millilter of sample solution.

(*b*) Calculate the ticarcillin or clavulanic acid anhydrous free acid content of the container as follows:

$$\text{Milligrams of anhydrous ticarcillin or clavulanic acid free acid per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ticarcillin or clavulanic acid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ticarcillin or clavulanic acid peak in the chromatogram of the ticarcillin or clavulanic acid working standard;

P_s=Anhydrous ticarcillian or clavulanic free acid activity in the ticarcillin-clavulanic acid working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of ticarcillin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 100 milligrams per milliliter.

[50 FR 34418, Aug. 20, 1985; 50 FR 42156, Oct. 18, 1985; 50 FR 43384, Oct. 25, 1985; 50 FR 45403, Oct. 31, 1985, as amended at 55 FR 11582, Mar. 29, 1990]

§440.290c Ticarcillin disodium and clavulanate potassium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ticarcillin disodium and clavulanate potassium injection is a frozen, aqueous, isoosmotic solution of ticarcillin disodium and clavulanate potassium with one or more suitable and harmless buffer substances. The ratio of ticarcillin to clavulanic acid is 30:1. Each milliliter contains ticarcillin disodium equivalent to 30 milligrams of ticarcillin and clavulanate potassium equivalent to 1 milligram of clavulanic acid. Its ticarcillin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of ticarcillin that it is represented to contain. Its clavulanate potassium content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of clavulanic acid that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.5 and not more than 7.5. It passes the identity test. The ticarcillin monosodium monohydrate used conforms to the standards prescribed by §440.91(a)(1). The clavulanate potassium used conforms to the standards prescribed by §455.15(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The ticarcillin monosodium monohydrate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(B) The clavulanate potassium used in making the batch for potency, moisture, pH, identity, and clavam-2-carboxylate content.

(C) The batch for ticarcillin content, clavulanic acid content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The ticarcillin monosodium monohydrate used in making the batch: 12 packages, each containing approximately 300 milligrams.

(B) The clavulanate potassium used in making the batch: 12 packages, each containing approximately 300 milligrams.

(C) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Ticarcillin and clavulanic acid contents.* Proceed as directed in §440.290b(b)(1), except use the thawed solution and prepare the sample solution and calculate the ticarcillin and clavulanic acid content as follows:

(i) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature. Dilute with diluent (described in §440.290b(b)(1)(i)(*c*)) to obtain a solution containing approximately 0.9 milligram of ticarcillin activity per milliliter (estimated). This solution will contain approximately 0.03 milligram of clavulanic acid per milliliter. Introduce the sample into the chromatograph in a timely manner.

(ii) *Calculations.* Calculate the ticarcillin or clavulanic acid concentration as follows:

$$\begin{array}{c}\text{Milligrams of ticarcillin}\\ \text{or clavulanic acid}\\ \text{activity per milliliter}\end{array} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ticarcillin or clavulanic acid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ticarcillin or clavulanic acid peak in the chromatogram of the ticarcillin or clavulanic acid working standard;

P_s=Ticarcillin or clavulanic acid activity in the ticarcillin-clavulanic acid working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 100 milligrams of ticarcillin per kilogram.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ticarcillin and clavulanic acid working standard.

[55 FR 5840, Feb. 20, 1990]

Subparts D–J [Reserved]

Subpart K—Bulk Drug Formulations for Repacking or for Manufacturing Use

§ 440.1080a Sterile penicillin G potassium buffered.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Penicillin G potassium, buffered, is a dry mixture of penicillin G potassium and the buffer sodium citrate in a quantity not less than 4.0 percent and not more than 5.0 percent by weight of its total solids. It may contain citric acid in a quantity not more than 0.15 percent of its total solids in place of a corresponding amount of sodium citrate. The sodium citrate and citric acid used in making the batch must conform to all U.S.P. specifications. It is so purified and dried that:

(i) Its potency is not less than 1,355 units and not more than 1,595 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH is not less than 6.0 and not more than 8.5.

(vii) Its penicillin G content is not less than 76.3 percent and not more than 89.8 percent.

(viii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, penicillin G content, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation*. Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures*. Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(c) Hydroxylamine colorimetric assay. Proceed as directed in § 436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

(7) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[42 FR 59872, Nov. 22, 1977, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

§ 440.1081a Sterile penicillin G sodium, buffered.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G sodium, buffered, is a dry mixture of penicillin G sodium and the buffer sodium citrate in a quantity not less than 4.0 percent and not more than 5.0 percent by weight of its total solids. It may contain citric acid in a quantity not more than 0.15 percent of its total solids in place of a corresponding amount of sodium citrate. The sodium citrate and citric acid used in making the batch must conform to all U.S.P. specifications. It is so purified and dried that:

(i) Its potency is not less than 1,420 units and not more than 1,667 units per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH is not less than 6.0 and not more than 7.5.

(vii) Its penicillin G content is not less than 80 percent and not more than 93.8 percent.

(viii) It is crystalline.

(ix) It passes the test for heat stability if it does not show a loss of more than 10 percent of its original potency.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, penicillin G content, crystallinity, and heat stability.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Assay for potency by any of the following methods; however, the results obtained from the iodometric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 unit of penicillin G per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, diluting an aliquot of the stock solution with solution 1 to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 20,000 units of penicillin G per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 60 milligrams per milliliter.

(7) *Penicillin G content.* Proceed as directed in § 436.316 of this chapter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Heat stability.* Proceed as directed in § 436.214 of this chapter.

[42 FR 59873, Nov. 22, 1977; 43 FR 2393, Jan. 17, 1978, as amended at 45 FR 22922, Apr. 4, 1980; 50 FR 19918, 19919, May 13, 1985]

PART 441—PENEM ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
441.20a Sterile imipenem monohydrate.

Subpart B [Reserved]

Subpart C—Injectable Dosage Forms

441.220 Imipenem monohydrate-cilastatin sodium injectable dosage forms.
441.220a Sterile imipenem monohydrate-cilastatin sodium.
441.220b Imipenem monohydrate-cilastatin sodium for injection.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§ 441.20a Sterile imipenem monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Imipenem monohydrate is the monohydrate form of [5*R*-[5α, 6α, (*R**)]]-6-(1-hydroxyethyl)-3-[[2-[(iminomethyl) amino]ethyl]thio]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic acid. It is a white to tan colored powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of imipenem per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its loss on drying is not less than 5.0 percent and not more than 8.0 percent.

(v) Its specific rotation in an aqueous solution containing 5 milligrams of imipenem per milliliter at 25 °C is +85° to +95° on an anhydrous basis.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, specific rotation, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using a column heater which will maintain a 50 °C column temperature, and ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octyl or octadecyl hydrocarbon bonded silicas, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Phosphate buffer, 0.001M.* Dissolve 272 milligrams of monobasic potassium phosphate in 1,800 milliliters of deionized water. Adjust the pH to 6.8 with 0.5*N* sodium hydroxide or dilute phosphoric acid. Dilute to 2,000 milliliters with deionized water and filter prior to use.

(*b*) *Mobile phase.* Dissolve 2.0 grams of 1-hexanesulfonic acid, sodium salt in 800 milliliters of phosphate buffer, 0.001*M*. Adjust the pH to 6.8 with 0.5*N* sodium hydroxide or dilute phosphoric acid and dilute to 1,000 milliliters with phosphate buffer, 0.001*M*. Filter and degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(*c*) *0.1 Percent bicarbonate solution.* Dissolve 50 milligrams of sodium bicarbonate in 40 milliliters of phosphate buffer, 0.001*M*, and dilute to 50 milliliters with phosphate buffer, 0.001*M*.

(*d*) *0.9 Percent saline solution.* Dissolve 9.0 grams of sodium chloride in 800 milliliters of deionized water and dilute to 1.0 liter with deionized water.

(ii) *Preparations of working standard and sample solutions*—(*a*) *Working standard solution.* Accurately weigh approximately 25 milligrams of the imipenem working standard into a 50-milliliter volumetric flask. Immediately prior to

analysis, add 10 milliliters of 0.9 percent saline solution and 1 milliliter of 0.1 percent bicarbonate solution. Add phosphate buffer, 0.001*M*, and shake until dissolved. Sonicate, if necessary, but for no longer than 1 minute. Dilute to volume with phosphate buffer, 0.001*M*, to obtain a solution containing approximately 500 micrograms of imipenem per milliliter. Mix well and inject immediately.

(*b*) *Sample solution*. Dissolve an accurately weighed portion (approximately 25 milligrams) of the sample with 10 milliliters of 0.9 percent saline solution and 1 milliliter of 0.1 percent bicarbonate solution in a 50-milliliter volumetric flask. Dilute the sample solution to volume with phosphate buffer, 0.001*M*, to obtain a solution containing 500 micrograms of imipenem per milliliter (estimated).

(iii) *System suitability requirements*—

(*a*) *Tailing factor*. The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(*b*) *Efficiency of the column*. The efficiency of the column (*n*) is satisfactory if it is greater than 600 theoretical plates for a 30-centimeter column.

(*c*) *Resolution*. The resolution (*R*) between the peaks for thienamycin and imipenem is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation (relative standard deviation)*. The coefficient of variation ($S_R$inpercent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*. Calculate the micrograms of imipenem per milligram of sample as follows:

$$\text{Micrograms of imipenem per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - L)}$$

where:

A_u=Area of the imipenem peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the imipenem peak in the chromatogram of the imipenem working standard;

P_s=Anhydrous imipenem activity in the imipenem working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

L=Percent loss on drying of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in §436.32(a) of this chapter, using a solution containing 5.0 milligrams of imipenem per milliliter, except inject 10 milliliters per kilogram of rabbit weight.

(4) *Loss on drying*. Proceed as directed in §436.200(i) of this chapter.

(5) *Specific rotation*. Dilute an accurately weighed sample with sufficient pH 7.0 phosphate buffer to give a concentration of approximately 5.0 milligrams of imipenem per milliliter. Proceed as directed in §436.210 of this chapter, using a 1.0-decimeter polarimeter tube. To prepare the pH 7.0 phosphate buffer, transfer 5 grams of monobasic potassium phosphate and 11 grams of dibasic potassium phosphate to a 1.0-liter volumetric flask. Dissolve and dilute to volume with distilled water.

(6) *Identity*. Proceed as directed in §436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(7) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[51 FR 11573, Apr. 4, 1986; 51 FR 16517, May 5, 1986, as amended at 55 FR 11582, Mar. 29, 1990; 59 FR 8133, Feb. 18, 1994]

Subpart B—[Reserved]

Subpart C—Injectable Dosage Forms

§441.220 Imipenem monohydrate-cilastatin sodium injectable dosage forms.

§441.220a Sterile imipenem monohydrate-cilastatin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Imipenem monohydrate-

cilastatin sodium is a dry mixture of imipenem monohydrate and cilastatin sodium packaged for dispensing. Its potency is satisfactory if it contains not less than 400 micrograms of imipenem and not less than 400 micrograms of cilastatin per milligram. Its imipenem content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of imipenem that it is represented to contain. Its cilastatin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cilastatin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 3.5 percent. When reconstituted as directed in the labeling, its pH is not less than 6.0 and not more than 7.5. The imipenem monohydrate used conforms to the standards prescribed by § 441.20a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The imipenem monohydrate used in making the batch for potency, sterility, pyrogens, loss on drying, specific rotation, identity, and crystallinity.

(B) The batch for imipenem potency, cilastatin potency, imipenem content, cilastatin content, sterility, pyrogens, loss on drying, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The imipenem monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Imipenem and cilastatin potency and content.* Determine the potency of the sample in micrograms per milligram of both imipenem and cilastatin and the milligrams of both imipenem and cilastatin per container. Proceed as directed in § 441.20a(b)(1), preparing the cilastatin reference standard solution, the sample solution and calculating the imipenem and cilastatin potency and content as follows:

(i) *Cilastatin reference standard.* Accurately weigh approximately 25 milligrams of the cilastatin reference standard into a 50-milliliter volumetric flask. Immediately prior to analysis, add 10 milliliters of a 0.9 percent saline solution and 1.0 milliliter of a 0.1 percent bicarbonate solution. Add phosphate buffer, 0.001*M*, and shake until dissolved. Sonicate, if necessary, but no longer than 1 minute. Dilute to volume with phosphate buffer, 0.001*M*, to obtain a solution containing approximately 500 micrograms of cilastatin per milliliter. Mix well and inject immediately.

(ii) *Preparation of sample solutions*—(A) *Imipenem and cilastatin potency (micrograms of imipenem and cilastatin per milligram).* Remove the metal seal from each of 10 containers and determine the gross weight in grams. Dissolve and wash out the entire contents of each container with a 0.9 percent saline solution into an appropriate size volumetric flask to give a concentration of 5 milligrams per milliliter each of imipenem and cilastatin. Further dilute with phosphate buffer, 0.001*M*, to obtain a solution containing 500 micrograms each of imipenem and cilastatin per milliliter (estimated). Wash each stopper and container with small quantities of acetone or methanol three times being careful not to wet the container labeling. Allow the containers to air dry about 3 hours or to constant weight. Weigh each container and stopper to determine tare weight in grams.

(B) *Imipenem and cilastatin content (milligrams of imipenem and cilastatin per container).* Reconstitute the sample as directed in the labeling, except use a 0.9 percent saline solution as the reconstituting fluid. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Accurately dilute

the solution thus obtained in a suitable volumetric flask with sufficient 0.9 percent saline solution to obtain a stock solution containing about 2,500 micrograms of imipenem and 2,500 micrograms of cilastatin per milliliter. Transfer a 10-milliliter aliquot of this solution to a 50-milliliter volumetric flask and dilute to volume with phosphate buffer, 0.001*M*, to obtain a solution containing 500 micrograms of imipenem and 500 micrograms of cilastatin per milliliter (estimated).

(iii) *Calculations*—(A) *Imipenem and cilastatin potency*. Calculate the micrograms of imipenem and cilastatin per milligram as follows:

$$\text{Milligrams of imipenem or cilastatin per milligram} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000 \times W_S}$$

where:

A_U=Area of the imipenem or cilastatin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the imipenem or cilastatin peak in the chromatogram of the imipenem or cilastatin working standard;

P_S=Anhydrous imipenem or cilastatin activity in the respective working standard solution in micrograms per milliliter;

d=Dilution factor of the 10 samples; and

W_S=Net contents of 10 containers in grams (gross weight of 10 containers in grams—tare weight of 10 containers in grams).

(B) *Imipenem and cilastatin content*. Calculate the imipenem or cilastatin content of the container as follows:

$$\text{Milligrams of imipenem or cilastatin per milligram} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000}$$

where:

A_U=Area of the imipenem or cilastatin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the imipenem or cilastatin peak in the chromatogram of the imipenem or cilastatin working standard;

P_S=Anhydrous imipenem or cilastatin activity in the imipenem or cilastatin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5.0 milligrams of imipenem per milliliter except inject 10 milliliters per kilogram of rabbit weight.

(4) *Loss on drying*. Proceed as directed in § 436.200(a) of this chapter.

(5) *pH*. Proceed as directed in § 436.202 of this chapter.

[58 FR 26670, May 4, 1993]

§ 441.220b Imipenem monohydrate-cilastatin sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Imipenem monohydrate-cilastatin sodium is a dry mixture of imipenem monohydrate, cilastatin sodium, and sodium bicarbonate packaged for dispensing. Its potency is satisfactory if it contains not less than 400 micrograms of imipenem and not less than 400 micrograms of cilastatin per milligram. Its imipenem content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of imipenem that it is represented to contain. Its cilastatin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cilastatin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 3.5 percent. When reconstituted as directed in the labeling, its pH is not less than 6.5 and not more than 8.5. The imipenem monohydrate used conforms to the standards prescribed by § 441.20a(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The imipenem monohydrate used in making the batch for potency, sterility, pyrogens, loss on drying, specific rotation, identity, and crystallinity.

(*b*) The batch for imipenem potency, cilastatin potency, imipenem content, cilastatin content, sterility, pyrogens, loss on drying, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research

(*a*) The imipenem used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Imipenem and cilastatin potency and content.* Determine the potency of the sample in micrograms per milligram of both imipenem and cilastatin and the milligrams of both imipenem and cilastatin per container. Proceed as directed in § 441.20a(b)(1) of this chapter, preparing the cilastatin reference standard solution, the sample solution and calculating the imipenem and cilastatin potency and content as follows:

(i) *Cilastatin reference standard.* Accurately weigh approximately 25 milligrams of the cilastatin reference standard into a 50–milliliter volumetric flask. Immediately prior to analysis, add 10 milliliters of 0.9 percent saline solution and 1 milliliter of 0.1 percent bicarbonate solution. Add phosphate buffer, 0.001*M*, and shake until dissolved. Sonicate, if necessary, but for no longer than 1 minute. Dilute to volume with phosphate buffer, 0.001*M*, to obtain a solution containing approximately 500 micrograms of cilastatin per milliliter. Mix well and inject immediately.

(ii) *Preparation of sample solutions*—(*a*) *Imipenem and cilastatin potency (micrograms of imipenem and cilastatin per milligram).* Remove the metal seal from each of 10 containers and determine gross weight in grams. Dissolve and wash out the entire contents of each container with 0.9 percent saline into an appropriate size volumetric flask to give a concentration of 5 milligrams per milliliter each of imipenem and cilastatin. Further dilute with phosphate buffer, 0.001 M, to obtain a solution containing 500 micrograms each of imipenem and cilastatin per milliliter (estimated). Wash each stopper and container with small quantities of acetone or methanol three times being careful not to wet the container labeling. Allow the containers to air dry about 3 hours or to constant weight. Weigh each container and stopper to determine tare weight in grams.

(*b*) *Imipenem and cilastatin content (milligrams of imipenem and cilastatin per container).* Reconstitute the sample as directed in the labeling, except use 0.9 percent saline solution as the reconstituting fluid. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Accurately dilute the solution thus obtained in a suitable volumetric flask with sufficient 0.9. percent saline solution to obtain a stock solution containing about 2,500 micrograms of imipenem and 2,500 micrograms of cilastatin per milliliter. Transfer a 10-milliliter aliquot of this solution to a 50-milliliter volumetric flask and dilute to volume with phosphate buffer, 0.001*M*, to obtain a solution containing 500 micrograms of imipenem and 500 micrograms of cilastatin per milliliter (estimated).

(iii) *Calculations*—(*a*) Calculate the micrograms of imipenem and cilastatin per milligram as follows:

$$\text{Micrograms of imipenem or cilastatin per milligram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times W_s}$$

where:

A_u=Area of the imipenem or cilastatin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the imipenem or cilastatin peak in the chromatogram of the imipenem or cilastatin acid working standard;

P_s=Anhydrous imipenem or cilastatin activity in the respective working standards solutions in micrograms per milliliter;

d=Dilution factor for the 10 samples; and

W_s=Net contents of 10 containers in grams (gross weight of 10 containers in grams–tare weight of 10 containers in grams).

(*b*) Calculate the imipenem or cilastatin content of the container as follows:

$$\text{Milligrams of imipenem or cilastatin per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the imipenem or cilastatin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the imipenem or cilastatin peak in the chromatogram of the imipenem or cilastatin working standard:

P_s=Anhydrous imipenem or cilastatin activity in the imipenem or cilastatin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 5.0 milligrams of imipenem per milliliter except inject 10 milliliters per kilogram of rabbit weight.

(4) *Loss on drying.* Proceed as directed in §436.200(a) of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter.

[51 FR 11573, Apr. 4, 1986; 51 FR 22275, June 19, 1986, as amended at 55 FR 11582, Mar. 29, 1990. Redesignated at 58 FR 26669, May 4, 1993]

PART 442—CEPHA ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
442.4 Cefaclor monohydrate.
442.6 Cefadroxil monohydrate.
442.7 Cefadroxil hemihydrate.
442.8a Sterile cefamandole nafate.
442.9a Sterile cefamandole sodium.
442.10 Cefazolin.
442.11a Sterile cefazolin sodium.
442.12 Cefoperazone sodium.
442.12a Sterile cefoperazone sodium.
442.13 Cefotaxime sodium.
442.13a Sterile cefotaxime sodium.
442.14 Cefoxitin sodium.
442.14a Sterile cefoxitin sodium.
442.15 Cefixime trihydrate.
442.16 Ceftazidime pentahydrate.
442.16a Sterile ceftazidime pentahydrate.
442.17 Ceftizoxime sodium.
442.17a Sterile ceftizoxime sodium.
442.18 Cefuroxime sodium.
442.18a Sterile cefuroxime sodium.
442.19 Cefuroxime axetil.
442.20a Sterile cefonicid sodium.
442.21 Cephaloglycin dihydrate.
442.22a Sterile cefmenoxime hydrochloride.
442.23a Sterile cephaloridine.
442.25a Sterile cephalothin sodium.
442.27 Cephalexin monohydrate.
442.28 Cephalexin hydrochloride monohydrate.
442.29a Sterile cephapirin sodium.
442.40 Cephradine.
442.40a Sterile cephradine.
442.41 Cephradine dihydrate.
442.50a Sterile ceforanide.
442.52 Cefotetan.
442.53a Sterile cefotetan disodium.
442.54 Cefpodoxime proxetil.
442.55 Ceftriaxone sodium.
442.55a Sterile ceftriaxone sodium.
442.58a Sterile cefotiam dihydrochloride.
442.60 Cefpiramide.
442.69 Cefmetazole.
442.70a Sterile cefmetazole sodium.
442.80 Cefprozil.

Subpart B—Oral Dosage Forms

442.104 Cefaclor monohydrate oral dosage forms.
442.104a Cefaclor monohydrate capsules.
442.104b Cefaclor monohydrate for oral suspension.
442.106 Cefadroxil monohydrate oral dosage forms.
442.106a Cefadroxil monohydrate capsules.
442.106b Cefadroxil monohydrate tablets.
442.106c Cefadroxil monohydrate for oral suspension.
442.107 Cefadroxil hemihydrate oral dosage forms.
442.107a Cefadroxil hemihydrate capsules.
442.107b Cefadroxil hemihydrate tablets.
442.115 Cefixime trihydrate oral dosage forms.
442.115a Cefixime trihydrate for oral suspension.
442.115b Cefixime trihydrate tablets.
442.119 Cefuroxime axetil oral dosage forms.
442.119a Cefuroxime axetil tablets.
442.119b Cefuroxime axetil for oral suspension.
442.121 Cephaloglycin dihydrate oral dosage forms.
442.121a Cephaloglycin dihydrate capsules.
442.121b Cephaloglycin dihydrate for oral suspension.
442.127 Cephalexin monohydrate oral dosage forms.
442.127a Cephalexin monohydrate tablets.
442.127b Cephalexin monohydrate capsules.
442.127c Cephalexin monohydrate for oral suspension.
442.128 Cephalexin hydrochloride monohydrate tablets.
442.140a Cephradine for oral suspension.
442.140b Cephradine capsules.
442.140c Cephradine tablets.
442.141 Cephradine dihydrate capsules.

442.154 Cefpodoxime proxetil oral dosage forms.
442.154a Cefpodoxime proxetil tablets.
442.154b Cefpodoxime proxetil granules for oral suspension.
442.180 Cefprozil oral dosage forms.
442.180a Cefprozil tablets.
442.180b Cefprozil for oral suspension.

Subpart C—Injectable Dosage Forms

442.208 Cefamandole nafate for injection.
442.209 Cefamandole sodium for injection.
442.211 Cefazolin sodium injectable dosage forms.
442.211a Sterile cefazolin sodium.
442.211b Cefazolin sodium injection.
442.212 Cefoperazone injectable dosage forms.
442.212a Sterile cefoperazone sodium.
442.212b Cefoperazone sodium injection.
442.213 Cefotaxime injectable dosage forms.
442.213a Sterile cefotaxime sodium.
442.213b Cefotaxime sodium injection.
442.214 Cefoxitin injectable dosage forms.
442.214a Sterile cefoxitin sodium.
442.214b Cefoxitin sodium injection.
442.216 Ceftazidime injectable dosage forms.
442.216a Ceftazidime pentahydrate for injection.
442.216b Ceftazidime sodium injection.
442.217 Ceftizoxime injectable dosage forms.
442.217a Sterile ceftizoxime sodium.
442.217b Ceftizoxime sodium injection.
442.218 Cefuroxime injectable dosage forms.
442.218a Sterile cefuroxime sodium.
442.218b Cefuroxime sodium injection.
442.220 Sterile cefonicid sodium.
442.222 Cefmenoxime hydrochloride for injection.
442.223 Sterile cephaloridine.
442.225 Cephalothin sodium injectable dosage forms.
442.225a Sterile sodium cephalothin.
442.225b Cephalothin sodium injection.
442.225c Cephalothin sodium for injection.
442.229 Sterile cephapirin sodium.
442.240 Cephradine injectable dosage forms.
442.240a Cephradine for injection.
442.240b Sterile cephradine.
442.250 Ceforanide for injection.
442.253 Cefotetan injectable dosage forms.
442.253a Sterile cefotetan disodium.
442.253b Cefotetan sodium injection.
442.255 Ceftriaxone injectable dosage forms.
442.255a Sterile ceftriaxone sodium.
442.255b Ceftriaxone sodium injection.
442.258 Cefotiam dihydrochloride for injection.
442.260 Cefpiramide sodium for injection.
442.270 Cefmetazole injectable dosage forms.
442.270a Sterile cefmetazole sodium.
442.270b Cefmetazole sodium injection.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19040, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§ 442.4 Cefaclor monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefaclor monohydrate is the monohydrate form of (6*R*, 7*R*)-7-[(*R*)-2-amino-2-phenylacetamido]-3-chloro-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 860 micrograms and not more than 1,050 micrograms of cefaclor per milligram on an ''as is'' basis.

(ii) Its moisture content is not less than 3.0 percent and not more than 8.0 percent.

(iii) Its pH in an aqueous suspension containing 25 milligrams per milliliter is not less than 3.0 and not more than 4.5.

(iv) It gives a positive identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution containing 1 milligram of cefaclor per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of cefaclor per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions

and calculate the cefaclor content as follows:

(*a*) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cefaclor working standard in sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter.

(*b*) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample in sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter.

(*c*) *Calculations.* Calculate the cefaclor content in micrograms per milligram as follows:

$$\text{Micrograms of cefaclor per milligram of sample} = \frac{A_u \times P_a}{A_s \times W_u}$$

where:

A_u = Absorbance of sample solution;
P_a = Potency of working standard solution in micrograms per milliliter;
A_s = Absorbance of working standard solution;
W_u = Milligrams of sample per milliliter of sample solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 25 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[46 FR 3832, Jan. 16, 1981]

§ 442.6 Cefadroxil monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil monohydrate is 7-[D-2-amino-2(*p*-hydroxyphenyl)acetamido] - 3 - methyl - 8 - oxo- 5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid monohydrate. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of cefadroxil per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.2 percent and not more than 6.0 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 4.0 and not more than 6.0.

(v) When calculated on an anhydrous basis, its absorptivity at 264 nanometers is not less than 95 percent and not more than 104 percent of that of the cefadroxil standard similarly treated and corrected for potency.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 20 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cefadroxil.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(*a*) *Preparation of working standard solutions.* Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to obtain a stock

solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter.

(*b*) *Preparation of sample solutions.* Dissolve an accurately weighed portion of the sample in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(*c*) Calculate the potency of the sample in micrograms per milligram as follows:

$$\begin{array}{c}\text{Micrograms}\\ \text{of cefadroxil}\\ \text{per milligram}\\ \text{of sample}\end{array} = \frac{A_u \times P_a \times 100}{A_s \times W_u \times (100 - m)}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution;
W_u=Milligrams of sample per milliliter of sampe solution;
m=Percent moisture in sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 50 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve accurately weighed portions of approximately 50 milligrams each of the sample and standard in 250 milliliters of distilled water. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with distilled water. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of each solution at 264 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

Percent relative absorptivity=[Absorbance of sample × milligrams standard × potency of standard in micrograms per milligram × 10]/[Absorbance of standard × milligrams sample × (100-*m*)]

where:

m=Percent moisture in the samples.

(6) *Identity.* Using the sample and working standard solutions prepared as described in paragraph (b)(5) of this section and a suitable spectrophotometer, record the ultraviolet spectrum from 220 to 340 nanometers. The spectrum of the sample compares qualitatively with that of the cefadroxil working standard.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 20977, May, 16, 1978; 43 FR 27180, June 23, 1978, as amended at 50 FR 19919, May 13, 1985]

§ 442.7 Cefadroxil hemihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil hemihydrate is 7-[D-2-amino-2(*p*-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of cefadroxil activity per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 2.4 percent and not more than 4.5 percent.

(iv) The pH of an aqueous solution containing 50 milligrams per milliliter is not less than 4.0 and not more than 6.0.

(v) When calculated on an anhydrous basis, its absorptivity at 264 nanometers is not less than 95 percent and not more than 104 percent of that of the cefadroxil standard similarly treated and corrected for potency.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefadroxil potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and

Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 20 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cefadroxil*. Proceed as directed in § 442.40(b)(1)(ii), except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(A) *Preparation of working standard solutions*. Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter.

(B) *Preparation of sample solutions*. Dissolve an accurately weighed portion of the sample in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(C) *Calculations*. Calculate the potency of the sample in micrograms per milligram as follows:

$$\text{Micrograms of cefadroxil per milligram} = \frac{A_U \times P_a \times 100}{A_s \times W_U \times (100 - m)}$$

where:

A_U=Absorbance of sample solution;

A_S=Absorbance of working standard solution;

P_a=Potency of working standard solution in micrograms per milliliter;

W_U=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 50 milligrams per milliliter.

(5) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve accurately weighed portions of approximately 50 milligrams each of the sample and standard in 250 milliliters of distilled water. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with distilled water. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of each solution at 264 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

Percent relative absorptivity = [Absorbance of sample X milligrams standard X potency of standard in micrograms per milligram X 10]/[Absorbance of standard X milligrams sample X (100-*m*)]

where:

m = Percent moisture in the samples.

(6) *Identity*. Using the sample and working standard solutions prepared as described in paragraph (b)(5) of this section and a suitable spectrophotometer, record the ultraviolet spectrum from 220 to 340 nanometers. The spectrum of the sample compares qualitatively with that of the cefadroxil working standard.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[59 FR 8857, Feb. 24, 1994]

§ 442.8a Sterile cefamandole nafate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cefamandole nafate is the sodium salt of 7-*D*-mandelamido-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylate formate (ester). It is so purified and dried that:

(i) Its potency is not less than 810 micrograms and not more than 1,000

micrograms of cefamandole per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2.0 percent.

(vi) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.5 and not more than 7.0.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing equal portions of approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Hydroxylamine colorimetric assay*. Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except use the cefamandole working standard.

(ii) *Polarographic assay*. Proceed as directed in § 436.324 of this chapter.

(iii) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a concentration of 1 milligram of cefamandole per milliliter (estimated). Hydrolyze this solution in a 37° C constant temperature water bath for 60 minutes. Further dilute a portion of the hydrolyzed solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 2.0 micrograms of cefamandole per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefamandole per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using the mineral oil mull prepared as described in paragraph (b)(2) of that section.

[47 FR 32708, June 1, 1982, as amended at 50 FR 19919, May 13, 1985]

§ 442.9a Sterile cefamandole sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cefamandole sodium is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(hydroxyphenylacetyl)amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt [6*R*-[6α, 7β(R*)]]-. It is so purified and dried that:

(i) Its cefamandole content is not less than 860 micrograms and not more than 1,000 micrograms of cefamandole per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 3.0 percent.

(vi) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.5 and not more than 7.0.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefamandole content, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing equal portions of approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Cefamandole content.* Proceed as directed in §436.324 of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 50 milligrams of cefamandole per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in §436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using the mineral oil mull prepared as described in paragraph (b)(2) of that section.

[47 FR 20756, May 14, 1982, as amended at 50 FR 19919, May 13, 1985]

§442.10 Cefazolin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefazolin is 3-[[(5-methyl-1, 3, 4-thiadiazol-2-yl)-thio]methyl]-7-[2-(1*H*-tetrazol-1-yl) acetamido]-3-cephem-4-carboxylic acid. It is so purified and dried that:

(i) Its cefazolin content is not less than 950 micrograms and not more than 1,030 micrograms of cefazolin per milligram calculated on an anhydrous basis.

(ii) Its moisture content is not more than 2 percent.

(iii) Its heavy metals content is not more than 20 parts per million.

(iv) It gives a positive identity test for cefazolin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefazolin content, moisture, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: Nine packages, each containing approximately 500 milligrams, and one package containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Cefazolin content.* Proceed as directed in §436.342 of this chapter.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Heavy metals.* Proceed as directed in §436.208 of this chapter.

(4) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefazolin working standard.

[48 FR 33479, July 22, 1983, as amended at 55 FR 11582, Mar. 29, 1990]

§442.11a Sterile cefazolin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile cefazolin sodium is the sodium salt of 3-[[(5-methyl - 1,3,4 - thiadiazol - 2 - yl) - thio]methyl] - 7 - [2 - (1H - tetrazol-1-yl)acetamido] - 3 - cephem - 4 - carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms and not more than 1050 micrograms of cefazolin per milligram calculated on an anhydrous basis. If it is packaged for dispensing, its cefazolin content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cefazolin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6 percent.

(vi) Its pH in an aqueous solution containing 100 milligrams of cefazolin per milliliter is not less than 4.5 and not more than 6.0.

(vii) The specific rotation in a 0.1*M* sodium bicarbonate solution containing 50 milligrams of cefazolin per milliliter at 25° C. is $-17° \pm 7°$ calculated on an anhydrous basis.

(viii) It gives a positive identity test for cefazolin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, specific rotation, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 9 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers, except if each contains less than 1.0 gram, a minimum of 24 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents. Dilute with sufficient solution 1 to give a stock solution of convenient concentration.

(ii) *Assay procedure.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 micrograms of cefazolin per milliliter (estimated).

(*b*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, preparing the working standard solution as follows: Dissolve an accurately weighed portion of approximately 30 milligrams of cefazolin working standard in 3 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 1 to the final concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefazolin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of cefazolin per milliliter.

(7) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using a solution containing 50 milligrams of cefazolin per milliliter in 0.1*M* sodium bicarbonate and a polarimeter tube 1.0 decimeter in length. Calculate the specific rotation on an anhydrous basis.

(8) *Identity.* Using a 0.002 percent solution of the sample in 0.1*M* sodium bicarbonate solution and a suitable spectrophotometer, record the ultraviolet spectrum from 220 to 340 nanometers. The spectrum compares qualitatively to that of the cefazolin working standard similarly tested.

[39 FR 19040, May 30, 1974, as amended at 42 FR 18059, Apr. 5, 1977; 50 FR 19919, May 13, 1985]

§ 442.12 Cefoperazone sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefoperazone sodium is the sodium salt of (6*R*, 7*R*)-7-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. It is a white to off-white crystalline powder or a lyophilized powder. It is so purified and dried that:

(i) Its cefoperazone content is not less than 870 micrograms and not more than 1,015 micrograms of cefoperazone per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 5.0 percent, except if it is the

lyophilized powder, its moisture content is not more than 2.0 percent.

(iii) The pH of an aqueous solution containing 250 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It passes the identity test if the retention times of the sample and working standard agree within ±3.0 percent.

(v) It is crystalline, except if it is the lyophilized powder.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefoperazone content, moisture, pH, identity, and crystallinity (if it is not the lyophilized powder).

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Cefoperazone content.* Proceed as directed in § 436.338 of this chapter.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams per milliliter.

(4) *Identity.* From the high-performance liquid chromatograms of the sample and the cefoperazone working standard determined as directed in paragraph (b)(1) of this section, calculate the adjusted retention times of the cefoperazone in the sample and standard solutions as follows:

Adjusted retention time of cefoperazone $= t - t_a$

where:

t = Retention time measured from point of injection into the chromatograph until the maximum of the cefoperazone sample or working standard peak appears on the chromatogram; and

t_a = Retention time measured from point of injection into the chromatograph until the maximum of nonretarded solute appears in the chromatogram.

The sample and the cefoperazone working standard should have corresponding adjusted cefoperazone retention times within ±3.0 percent.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[51 FR 36688, Oct. 15, 1986, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.12a Sterile cefoperazone sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile cefoperazone sodium is the sodium salt of (6*R*, 7*R*)-7-[(*R*)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. It is a white to off-white crystalline powder or it may be a lyophilized powder. It is so purified and dried that:

(i) If the cefoperazone sodium is not packaged for dispensing, its cefoperazone content is not less than 870 micrograms and not more than 1,015 micrograms of cefoperazone per milligram on an anhydrous basis. If the cefoperazone sodium is packaged for dispensing, its cefoperazone content is not less than 870 micrograms and not more than 1,015 micrograms of cefoperazone per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefoperazone that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 5.0 percent, except if it is the lyophilized powder, its moisture content is not more than 2.0 percent.

(v) Its pH in an aqueous solution containing 250 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(vi) It passes the identity test if the retention times of the sample and working standard agree within ±3 percent.

(vii) It is crystalline, except if it is the lyophilized powder, it is not crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefoperazone content, sterility, pyrogens, moisture, pH, identity, and crystallinity (if it is not the lyophilized powder).

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for manufacturing use:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefoperazone content.* Proceed as directed in §436.338 of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 10 milligrams of cefoperazone per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 250 milligrams per milliliter.

(6) *Identity.* From the high-pressure liquid chromatograms of the sample and the cefoperazone working standard determined as directed in paragraph (b)(1) of this section, calculate the adjusted retention times of the cefoperazone in the sample and standard solutions as follows:

Retention time of cefoperazone$=t_s-t_u$

where:

t_s=Retention time of working standard measured from point of injection into the chromatograph until the peak maximum appears on the chromatogram; and

t_u=Retention time of sample measured from point of injection into the chromatograph until the peak maximum appears on the chromatogram.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[48 FR 790, Jan. 7, 1983; 43 FR 7439, Feb. 22, 1983; 48 FR 28250, June 21, 1983, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.13 Cefotaxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotaxime sodium is the sodium salt of 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo,[6*R*-[6 *alpha*, 7 *beta*(*Z*)]]-. It is so purified and dried that:

(i) Its potency is not less than 855 micrograms and not more than 1,002 micrograms of cefotaxime per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 6.0 percent.

(iii) Its pH in an aqueous solution is not less than 4.5 and not more than 6.5.

(iv) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research; 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution

with solution 1 to the reference concentration of 2.0 micrograms of cefotaxime per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii), except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(*a*) *Preparation of the working standard solution.* Dissolve and dilute an accurately weighed portion of the cefotaxime working standard in sufficient distilled water to obtain a concentration of 1 milligram of cefotaxime per milliliter.

(*b*) *Preparation of sample solution.* Dissolve and dilute an accurately weighed portion of the sample in sufficient distilled water to obtain a concentration of 1 milligram of cefotaxime per milliliter (estimated).

(*c*) *Calculation.* Calculate the cefotaxime content in micrograms per milligram as follows:

$$\text{Micrograms of cefotaxime per milligram of sample} = \frac{A_u \times P_a}{A_s \times W_u}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard solution in micrograms per milliliter;
A_s=Absorbance of working standard solution; and
W_u=Milligrams of sample per milliliter of sample solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.323 of this chapter, except prepare spotting solutions as follows: Prepare solutions of the sample and working standard, each containing 1 milligram of cefotaxime per milliliter in distilled water.

[50 FR 45109, Oct. 30, 1985, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.13a Sterile cefotaxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotaxime sodium is the sodium salt of 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-,[6*R*-[6α, 7β(*Z*)]]-. It is so purified and dried that:

(i) Its potency is not less than 855 micrograms and not more than 1,002 micrograms of cefotaxime per milligram on an anhydrous basis. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefotaxime that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in an aqueous solution is not less than 4.5 and not more than 6.5.

(vii) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 1 gram.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately

weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2.0 micrograms of cefotaxime per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(*a*) *Preparation of the working standard solution.* Dissolve and dilute an accurately weighed portion of the cefotaxime working standard in sufficient distilled water to obtain a concentration of 1 milligram of cefotaxime per milliliter.

(*b*) *Preparation of sample solution.* Dissolve and dilute an accurately weighed portion of the sample in sufficient distilled water to obtain a concentration of 1 milligram of cefotaxime per milliliter (estimated).

(*c*) *Calculations*—(*1*) Calculate the cefotaxime content in micrograms per milligram as follows:

$$\text{Micrograms of cefotaxime per milligram of sample} = \frac{A_u \times P_a}{A_s \times W_u}$$

where:

A_u = Absorbance of sample solution;
P_a = Potency of working standard solution in micrograms per milliliter;
A_s = Absorbance of working standard solution;
W_u = Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the cefotaxime content of the single-dose vial as follows:

$$\text{Milligrams of cefotaxime per single-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u = Absorbance of sample solution;
P_a = Potency of working standard solution in micrograms per milliliter;
A_s = Absorbance of working standard solution;
d = Dilution factor of the sample.

(*3*) Calculate the cefotaxime content of the multiple-dose vial as follows:

$$\text{Milligrams of cefotaxime per multiple-dose vial} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u = Absorbance of sample solution;
P_a = Potency of working standard solution in micrograms per milliliter;
A_s = Absorbance of working standard solution;
d = Dilution factor of the sample;
n = Volume of sample solution assayed.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefotaxime per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity.* Proceed as directed in § 436.323 of this chapter, except prepare spotting solutions as follows: Prepare solutions of the sample and working standard, each containing 1 milligram of cefotaxime per milliliter in distilled water.

[46 FR 25606, May 8, 1981, as amended at 50 FR 19919, May 13, 1985]

§ 442.14 Cefoxitin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefoxitin sodium is the sodium salt of 3-(hydroxymethyl)-7α-methoxy-8-oxo-7-[2-(2-thienyl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic

acid carbamate (ester). It is so purified and dried that:

(i) Its cefoxitin content is not less than 850 micrograms and not more than 1,000 micrograms of cefoxitin per milligram.

(ii) Its moisture content is not more than 2.0 percent.

(iii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.2 and not more than 7.0.

(iv) It gives a positive identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefoxitin content, moisture, pH, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Cefoxitin content.* Proceed as directed in § 436.347 of this chapter, preparing the working standard and sample solutions and calculating the cefoxitin content as follows:

(i) *Working standard solution.* Dissolve an accurately weighed portion of the cefoxitin working standard with water to obtain a solution containing 1 milligram of cefoxitin per milliliter.

(ii) *Sample solution.* Dissolve an accurately weighed portion of the sample with water to obtain a solution containing 1 milligram of cefoxitin per milliliter (estimated).

(iii) *Calculations.* Calculate the micrograms of cefoxitin per milligram of sample as follows:

$$\text{Micrograms of cefoxitin per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the cefoxitin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefoxitin peak in the chromatogram of the cefoxitin working standard;

P_s=Cefoxitin activity in the cefoxitin working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section, except add about 25 milliliters of methanol in lieu of solvent A to a dry titrating vessel and proceed as directed in titration procedure 1.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.326 of this chapter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[49 FR 47827, Dec. 7, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.14a Sterile cefoxitin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefoxitin sodium is the sodium salt of 3-(hydroxymethyl)-7α-methoxy-8-oxo-7-[2-(2-thienyl) acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid carbamate (ester). It is so purified and dried that:

(i) Its potency is not less than 850 micrograms and not more than 1,000 micrograms of cefoxitin per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefoxitin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2.0 percent.

(vi) Its pH in an aqueous solution is not less than 4.2 and not more than 7.0.

(vii) It gives a positive identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 1 gram.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.347 of this chapter, preparing the working standard and sample solutions and calculating the cefoxitin content as follows:

(i) *Working standard solution*. Dissolve an accurately weighed portion of the cefoxitin working standard with distilled water to obtain a solution containing 1 milligram of cefoxitin per milliliter.

(ii) *Sample solutions*. Dissolve an accurately weighed portion of the sample with distilled water to obtain a solution containing 1 milligram of cefoxitin per milliliter (estimated); and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to obtain a solution containing 1 milligram of cefoxitin per milliliter (estimated).

(iii) *Calculations*—(*a*) Calculate the cefoxitin content in micrograms per milligram as follows:

$$\text{Micrograms of cefoxitin per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the cefoxitin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefoxitin peak in the chromatogram of the cefoxitin working standard;

P_s=Cefoxitin activity in the cefoxitin working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution (estimated).

(*b*) Calculate the cefoxitin content of the vial as follows:

$$\text{Milligrams of cefoxitin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefoxitin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefoxitin peak in the chromatogram of the cefoxitin working standard;

P_s=Cefoxitin activity in the cefoxitin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefoxitin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section, except add about 25 milliliters of methanol in lieu of solvent A to a dry titrating vessel and proceed as directed in titration procedure 1.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity*. Proceed as directed in § 436.326 of this chapter, preparing the sample as follows: Prepare a solution containing about 2.5 milligrams of cefoxitin per milliliter in distilled water.

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[44 FR 10374, Feb. 20, 1979, as amended at 50 FR 19919, May 13, 1985; 51 FR 27532, Aug. 1, 1986]

§442.15 Cefixime trihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefixime trihydrate is the trihydrate form of [6*R*-[6 α, 7B(*Z*)]]-7-[[(2-amino-4-thiazolyl) [(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,030 micrograms of cefixime activity per milligram, on an anhydrous basis.

(ii) Its moisture content is not less than 9.0 percent and not more than 12.0 percent.

(iii) The pH of an aqueous solution containing the equivalent of 0.7 milligram per milliliter is not less than 2.6 and not more than 4.1.

(iv) It is crystalline.

(v) The specific rotation in a 2.0 percent sodium bicarbonate solution containing 10.0 milligrams of cefixime per milliliter at 25 °C is between −75° and −88° calculated on an anhydrous basis.

(vi) It gives a positive identity test for cefixime.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results for tests and assays on the batch for potency, moisture, pH, crystallinity, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.216 of this chapter, using an ultraviolet detection system operating at a wavelength of 254 nanometers, and a column (typically 3 centimeters × 4.6 millimeters) packed with a 3-micron octadecyl hydrocarbon bonded silica or equivalent at ambient temperature. Reagents, working standard, test and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Phosphoric acid solution.* Add 10 milliliters of concentrated phosphoric acid to 90 milliliters of water.

(B) *Tetrabutylammonium hydroxide solution.* Dilute 25 milliliters of 0.4*M* tetrabutylammonium hydroxide solution to 1,000 milliliters with water. Adjust the pH to 7.0 with phosphoric acid solution.

(C) *Mobile phase.* Add 775 milliliters of the tetrabutylammonium hydroxide solution to 225 milliliters of acetonitrile. Filter the mobile phase through a suitable glass filter or equivalent which is capable of removing particulate contamination greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(D) *0.1M Phosphate buffer, pH 7.0.* Add 6.8 milliliters of concentrated phosphoric acid to 300 milliliters of water. Adjust the pH to 7.0 with 10*N* sodium hydroxide. Dilute to 1,000 milliliters with water.

(ii) *Preparation of working standard, test and sample solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of the cefixime standard with sufficient 0.1*M* phosphate buffer, pH 7.0, to obtain a solution of known concentration containing approximately 2 milligrams of cefixime activity per milliliter. Further dilute quantitatively to a final concentration of 0.2 milligram of cefixime activity per milliliter in 0.1 *M* phosphate buffer, pH 7.0. Prepare the working standard solution just prior to its introduction into the chromatograph.

(B) *System suitability test solution.* Dissolve an accurately weighed portion of cefixime working standard in distilled water to obtain a solution containing approximately 1.0 milligram of cefixime activity per milliliter. Heat this solution at 95 °C (in an oil bath) for 45 minutes. This procedure allows the (E)-isomer of cefixime to be generated *in situ.* Prepare the test solution

just prior to its introduction into the chromatograph.

(C) *Sample solution*. Accurately weigh approximately 100 milligrams of the sample into a 50-milliliter volumetric flask. Dilute to volume with 0.1*M* phosphate buffer, pH 7.0, to obtain a stock solution containing approximately 2 milligrams of cefixime activity per milliliter. Mix well. Immediately prior to chromatography, further dilute 10 milliliters of stock solution to 100 milliliters with 0.1 *M* phosphate buffer, pH 7.0 to obtain a solution containing 0.2 milligram of cefixime activity per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Asymmetry factor*. Calculate the asymmetry factor (A_s), measure data point that is 10 percent of the cefixime peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:
a=Horizontal distance from point of ascent to point of maximum peak height; and
b=horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 0.85 and not more than 1.5.

(B) *Efficiency of the column*. From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) for the cefixime peak as follows:

$$(h_r) = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:
L=Length of the column in centimeters;
n=number of theoretical plates; and
(d_p)=Average diameter of the particles in the column in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15 for the cefixime peak.

(C) *Resolution*. The resolution (R) between the peak for cefixime and the peak for the (E)-isomer of cefixime (generated *in situ*) is not less than 1.1.

(D) *Coefficient of variation (relative standard deviation*). The coefficient of variation (S_R) in percent) of five replicate injections is satisfactory if not more than 2.0 percent

(E) Capacity factor (k). Calculate the capacity factor (k) for cefixime as follows:

$$(k) = \frac{t_r - t_m}{t_m}$$

where:
t_r=Retention time of solute; and
t_m=Retention time of solvent or unretained substance, calculated as follows:

$$t_m = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:
D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (k) for cefixime is satisfactory if it is not less than 5 and not more than 11.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided that the system suitability parameters are met. However, the sample preparation described in paragraph (b)(1)ii)(C) of this section should not be changed.

(iv) *Calculations*. Calculate the micrograms of cefixime anhydrous free acid per milligram as follows:

$$\begin{matrix}\text{Micrograms of}\\ \text{cefixime}\\ \text{per milligram}\end{matrix} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:
A_u=Area of the cefixime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the cefixime peak in the chromatogram of the cefixime working standard;
P_s=Cefixime activity in the cefixime working standard solution in micrograms per milliliter;
C_u=Milligrams of sample per milliliter of sample solution; and
m=Percent moisture content of the sample.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 0.7 milligram per milliliter.

(4) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

(5) *Specific rotation*. Dissolve and dilute an accurately weighed sample with sufficient 2 percent sodium bicarbonate to obtain a concentration of approximately 10 milligrams of cefixime per milliliter. Proceed as directed in §436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(6) *Identity*. Proceed as directed in §436.211 of this chapter, using a potassium bromide disc containing 0.5 percent of cefixime. Dissolve 5 to 6 milligrams of cefixime in 2 milliliters of methanol. Triturate to insure solution. Evaporate the solvent to dryness and using the dried sample, prepare the potassium bromide disc.

[53 FR 24257, June 28, 1988; 53 FR 26712, July 14, 1988; 54 FR 47205, Nov. 13, 1989]

§442.16 Ceftazidime pentahydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ceftazidime pentahydrate is pyridinium, 1-[[7-[[(2-amino-4-thiazolyl)[1-carboxy-1-methylethoxy)imino]acetyl]-amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-, hydroxide, inner salt, [6*R*-[6α,7β(*Z*)]]-, pentahydrate. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of ceftazidime activity per milligram on an anhydrous basis.

(ii) Its loss on drying is not less than 13.0 percent and not more than 15.0 percent.

(iii) The pH of an aqueous solution containing 5 milligrams of ceftazidime per milliliter is not less than 3.0 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for ceftazidime.

(vi) Its high molecular weight polymer content is not more than 0.05 percent.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, crystallinity, identity, and high molecular weight polymer content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §442.16a(b)(1).

(2) *Loss on drying*. Proceed as directed in §436.200(a) of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 5 milligrams of ceftazidime per milliliter.

(4) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

(5) *Identity*. The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ceftazidime working standard.

(6) *High molecular weight polymer content*. Proceed as directed in §442.16a(b)(8).

[54 FR 40652, Oct. 3, 1989]

§442.16a Sterile ceftazidime pentahydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile ceftazidime pentahydrate is pyridinium, 1-[[7-[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-, hydroxide, inner salt, [6*R*-[6α, 7β(*Z*)]]-, pentahydrate. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of ceftazidime activity per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its loss on drying is not less than 13.0 and not more than 15.0 percent.

(v) Its pH in an aqueous solution containing 5 milligrams of ceftazidime per milliliter is not less than 3.0 and not more than 4.0.

(vi) It is crystalline.

(vii) It gives a positive identity test for ceftazidime.

(viii) Its high molecular weight polymer content is not more than 0.05 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain.

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, crystallinity, identity, and high molecular weight polymer content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: One package containing approximately 6 grams of a composite sample.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.356 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as hexyl, octyl, or octaldecyl hydrocarbon bonded silicas, a flow rate of 2.0 milliliters per minute, and a known injection volume of 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Phosphate buffer, pH 7.0.* Dissolve 42.59 grams of sodium phosphate, dibasic anhydrous and 27.22 grams of potassium phosphate, monobasic, in water and dilute to 1,000 milliliters.

(*b*) *Mobile phase.* Mix 40 milliliters of acetonitrile and 200 milliliters of phosphate buffer, pH 7.0, and dilute to 2,000 milliliters with water. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions*—(*a*) *Working standard solution.* Accurately weigh ceftazidime working standard equivalent to approximately 100 milligrams of the ceftazidime activity into a 100-milliliter volumetric flask containing 10 milliliters of phosphate buffer, pH 7.0. Shake until dissolved. Dilute to volume with water to obtain a stock solution containing approximately 1,000 micrograms of ceftazidime activity per milliliter. Mix well. Immediately prior to chromatography, further dilute 5 milliliters of stock solution to 50 milliliters with water to obtain a solution containing 100 micrograms of ceftazidime activity per milliliter.

(*b*) *Sample solution.* Accurately weigh approximately 115 milligrams of the sample into a 100-milliliter volumetric flask containing 10 milliliters of phosphate buffer, pH 7.0. Shake until dissolved. Dilute to volume with water to obtain a stock solution containing approximately 1,000 micrograms of ceftazidime per milliliter. Mix well. Immediately prior to chromatography, further dilute 5 milliliters of stock solution to 50 milliliters with water to obtain a solution containing 100 micrograms of ceftazidime activity per milliliter (estimated).

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 1.5 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1,500 theoretical plates.

(*c*) *Resolution.* The resolution (R) between the peak for ceftazidime and its nearest eluting impurity is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 1.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.356(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility

and resolution are provided comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations.* Calculate the micrograms of ceftazidime per milligram of sample as follows:

$$\text{Micrograms of ceftazidime per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u (100 - m)}$$

where:

A_u=Area of the ceftazidime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftazidime peak in the chromatogram of the ceftazidime working standard;

P_s=Ceftazidime activity in the ceftazidime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent loss on drying content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except dissolve the sample in approximately 200 milliliters of diluting fluid H.

(3) *Pyrogens.* Proceed as directed in § 436.32(i) of this chapter, using a solution containing 80 milligrams of ceftazidime per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams of ceftazidime per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ceftazidime working standard.

(8) *High molecular weight polymer content.* Proceed as directed in § 436.360 of this chapter, using a constant temperature between 20 and 25 °C, an ultraviolet detection system operating at a wavelength of 235 nanometers, a column packed with a hydrophilic gel for gel permeation chromatography (such as Fractogel TSK HW–40(F), Merck) or equivalent, a flow rate of 1.0 milliliter per minute, and a known injection volume of 100 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Mobile phase.* Adjust a 0.1*M* solution of potassium phosphate, dibasic, to pH 7.0±0.1 with phosphoric acid.

(*b*) *Blue dextran system suitability test solution.* Prepare a solution in mobile phase containing 100 micrograms per milliliter of blue dextran (with a mean molecular weight of approximately 2,000,000).

(ii) *Preparation of working standard and sample solutions*—(*a*) *Working standard solution.* Accurately weigh high molecular weight polymer working standard equivalent to approximately 400 micrograms of high molecular weight polymer into a 100-milliliter volumetric flask and add 80 milliliters of mobile phase. Shake until dissolved and dilute to volume with mobile phase to obtain a solution containing approximately 4 micrograms of high molecular weight polymer per milliliter. Store the solution at ambient temperature and inject into the chromatograph within one hour of preparation.

(*b*) *Sample solution.* Accurately weigh approximately 400 milligrams of the sample into a 100-milliliter volumetric flask and add 80 milliliters of mobile phase. Shake until dissolved, dilute to volume with mobile phase, and immediately inject the solution into the liquid chromatograph.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5 for blue dextran.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates for blue dextran.

(*c*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections of blue dextran is satisfactory if it is not more than 4 percent.

If the system suitability requirements have been met, then proceed as described in § 436.360(b) of this chapter.

(iv) *Calculations.* Calculate the percent of high molecular weight polymer content as follows:

$$\text{High molecular weight polymer content in percent} = \frac{H_u \times P_s \times 0.1}{H_s \times C_u}$$

where:

H_u=Height of the high molecular weight polymer peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

H_s=Mean height of the high molecular weight polymer peaks in the chromatograms of the high molecular weight polymer working standard;

P_s=High molecular weight polymer content of the high molecular weight polymer working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

[50 FR 48399, Nov 25, 1985; 50 FR 53308, Dec. 31, 1985; 51 FR 2478, Jan. 17, 1986, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.17 Ceftizoxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftizoxime sodium is the sodium salt of [6*R*-[6α, 7β(*Z*)]]-7-[[(2,3-dihydro-2-imino-4-thiazolyl) (methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its ceftizoxime content is not less than 850 micrograms and not more than 995 micrograms of ceftizoxime per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 8.5 percent.

(iii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 6.0 and not more than 8.0

(iv) It gives a positive identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for ceftizoxime content, moisture, pH, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Ceftizoxime content.* Proceed as directed in § 436.345 of this chapter, preparing the sample solution and calculating the ceftizoxime content as described in paragraphs (e)(1) and (g)(1), respectively, of that section.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(4) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the ceftizoxime working standard.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[49 FR 49285, Dec. 19, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.17a Sterile ceftizoxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftizoxime sodium is the sodium salt of [6*R*-[6α,7β(*Z*)]]-7-[[(2,3-dihydro-2-imino-4-thiazolyl) (methoxyimino) acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) If the ceftizoxime is not packaged for dispensing, its ceftizoxime content is not less than 850 micrograms and not more than 995 micrograms of ceftizoxime per milligram on an anhydrous basis. If the ceftizoxime is packaged for dispensing, its ceftizoxime content is not less than 850 micrograms and not more than 995 micrograms of ceftizoxime per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 115 percent of the number of milligrams of ceftizoxime that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 8.5 percent.

(v) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for ceftizoxime content, sterility, pyrogens, moisture, pH, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing at least 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers; or if each container contains less than 1 gram of ceftizoxime, a minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ceftizoxime content.* Proceed as directed in § 436.345 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of ceftizoxime per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Identity.* From the high-pressure liquid chromatograms of the sample and the ceftizoxime working standard determined as directed in paragraph (b)(1) of this section, calculate the adjusted retention times of the ceftizoxime in the sample and standard solutions as follows:

$$\text{Adjusted retention time of ceftizoxime} = t - t_a$$

where:

t=Retention time measured from point of injection into the chromatograph until the maximum of the ceftizoxime sample or working standard peak appears on the chromatogram; and

t_a=Retention time measured from point of injection into the chromatograph until the maximum of nonretarded solute appears in the chromatogram.

The sample and the ceftizoxime working standard should have corresponding adjusted ceftizoxime retention times.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[48 FR 46271, Oct. 12, 1983; 48 FR 49656, Oct. 27, 1983, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.18 Cefuroxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime sodium is the sodium salt of (6*R*,7*R*)-3-carbamoyloxymethyl-7-[(2*Z*)-2-(2-furyl)-2-methoxyiminoacetamido]cepha-3-em-4-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 855 micrograms and not more than 1,000 micrograms of cefuroxime activity per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 3.5 percent.

(iii) The pH of an aqueous solution containing 100 milligrams of cefuroxime per milliliter is not less than 6.0 and not more than 8.5.

(iv) It gives a positive identity test for cefuroxime.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 1 gram.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 442.343.

(2) *Moisture.* Proceed as directed in § 436.18a(b)(4) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of cefuroxime per milliliter.

(4) *Identity.* Proceed as directed in § 442.18a(b)(6).

[54 FR 40654, Oct. 3, 1989; 54 FR 50686, Dec. 8, 1989]

§ 442.18a Sterile cefuroxime sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime sodium is the sodium salt of (6*R*, 7*R*)-3-carbamoyloxy-methyl-7-[(2*Z*)-2-(2-furyl)-2-methoxyiminoacetamido] cepha-3-em-4-carboxylic acid. It is so purified and dried that:

(i) If the cefuroxime is not packaged for dispensing, its cefuroxime content is not less than 855 micrograms and not more than 1,000 micrograms of cefuroxime per milligram on an anhydrous basis. If the cefuroxime is packaged for dispensing, its cefuroxime content is not less than 855 micrograms and not more than 1,000 micrograms of cefuroxime per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefuroxime that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 3.5 percent.

(v) Its pH in an aqueous solution is not less than 6.0 and not more than 8.5.

(vi) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefuroxime content, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 20 packages, each containing approximately 1 gram.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefuroxime content.* Proceed as directed in § 436.343 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefuroxime per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Identity.* From the high-pressure liquid chromatograms of the sample and the cefuroxime working standard determined as directed in paragraph (b)(1) of this section, calculate the adjusted retention times of the cefuroxime in the sample and standard solutions as follows:

Adjusted retention time of cefuroxime $= t - t_a$

where:

t = Retention time measured from point of injection into the chromatograph until the maximum of the cefuroxime sample or working standard peak appears on the chromatogram; and

t_a = Retention time measured from point of injection into the chromatograph until the maximum of nonretarded solute appears in the chromatogram.

The sample and the cefuroxime working standard should have corresponding adjusted cefuroxime retention times.

[48 FR 38461, Aug. 24, 1983, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.19 Cefuroxime axetil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime axetil is an amorphous mixture of the diastereoisomers of 5-thia-1-azabicyclo[4.2.0]oct-

2-ene-2-carboxylic acid, 3-[[(aminocarbonyl)oxy]methyl]-7-[[2-furanyl(methoxyimino)acetyl]amino]-8-oxo-, 1-(acetyloxy)ethyl ester, [6*R*-[6 *alpha*, 7 *beta* (*Z*)]]-. It is so purified and dried that:

(i) Its potency is not less than 745 micrograms and not more than 875 micrograms of cefuroxime per milligram on an anhydrous basis. The ratio of isomer A to total isomer content is not less than 0.48 and not more than 0.55.

(ii) Its moisture content is not more than 1.5 percent.

(iii) It is amorphous and not crystalline.

(iv) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Request for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefuroxime potency, isomer A ratio, moisture, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 278 nanometers, a 25-centimeter by 4.6-millimeter column packed with methyl silane bonded silica 5 micrometers in particle size, a flow rate of 1 milliliter per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.2M Ammonium phosphate solution.* Transfer 23.0 grams of ammonium dihydrogen phosphate to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water. Mix well.

(B) *Mobile phase.* Transfer 380 milliliters of methanol to a 1-liter volumetric flask and dilute to volume with 0.2*M* ammonium phosphate solution.

(C) *Internal standard solution.* Prepare a solution containing 5.4 milligrams of acetanilide per milliliter in methanol.

(D) *System suitability test solution.* Mix 10.0 milliliters of a solution containing 1.2 milligrams of cefuroxime axetil working standard per milliliter in methanol with 5.0 milliliters of internal standard solution, 2.0 milliliters of a solution containing 0.3 milligram of an authentic sample of (*RS*)-1-acetoxyethyl (6*R*, 7*R*)-3-carbamoyloxymethyl-7-[(2′*Z*)-2-(fur-2-yl)-2-methoxy-iminoacetamido]ceph-2-em-4-carboxylate (delta-2 isomers of cefuroxime axetil) per milliliter in methanol and 1.8 milliliters of methanol. Dilute to 50 milliliters with 0.2*M* ammonium phosphate solution.

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve approximately 30 milligrams of the cefuroxime axetil working standard, accurately weighed, in methanol and dilute to 25 milliliters with methanol. Immediately transfer 10.0 milliliters of the working standard solution to a 50-milliliter volumetric flask. Add 5.0 milliliters of internal standard solution and 3.8 milliliters of methanol, and dilute to volume with 0.2*M* ammonium phosphate soluton to obtain a solution containing 0.2 milligram of cefuroxime activity per milliliter. Store the solution under refrigeration no more than 8 hours.

(B) *Sample solution.* Dissolve approximately 30 milligrams of the sample, accurately weighed, in methanol and dilute to 25 milliliters with methanol. Immediately transfer 10.0 milliliters of the sample solution to a 50-milliliter volumetric flask. Add 5.0 milliliters of internal standard solution and 3.8 milliliters of methanol, and dilute to volume with 0.2*M* ammonium phosphate solution to obtain a solution containing 0.2 milligram of cefuroxime activity per milliliter (estimated). Store the solution under refrigeration no more than 8 hours.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory for isomer A if it is not more than 1.5 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory

for isomer A if it is greater than 3,000 theoretical plates.

(C) *Resolution*. The resolution (R) between isomer A and isomer B of cefuroxime axetil is satisfactory if it is not less than 1.5 and the resolution (R) between isomer A and the delta-2 isomers of cefuroxime axetil is satisfactory if it is not less than 1.5.

(D) *Coefficent of variation*. The coefficient of variation ($S_R$in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolutiona recomparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations*—(A) Calculate the micrograms of cefuroxime per milligram of sample as follows:

$$\text{Micrograms of cefuroxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u = Sum of the peak height of the cefuroxime axetil sample isomer A and isomer B peaks/Peak height of the internal standard;

R_s = Sum of the peak heights of the cefuroxime axetil working standard isomer A and isomer B peaks/Peak height of the internal standard;

P_s = Cefuroxime activity in the cefuroxime axetil working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(B) Calculate the ratio of isomer A to total isomer content as follows:

$$\text{Ratio of isomer A to isomer content} = \frac{\text{Peak height of isomer A peak}}{\text{Peak height of isomer A peak} + \text{Peak height of isomer B peak}}$$

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section.

(3) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter, except that the particles do not reveal the phenomena of birefringence and extinction positions on revolving the microscope stage.

(4) *Identity*. Proceed as directed in § 436.211 of this chapter, using the mineral oil mull prepared as described in paragraph (b)(2) of that section.

[52 FR 42432, Nov. 5, 1987; 52 FR 43966, Nov. 17, 1987; 52 FR 45528, Nov. 30, 1987, as amended at 54 FR 47351, Nov. 14, 1989; 55 FR 11583, Mar. 29, 1990]

§ 442.20a Sterile cefonicid sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cefonicid sodium is a white to off-white lyophilized powder. It is so purified and dried that:

(i) If the cefonicid sodium is not packaged for dispensing, its cefonicid content is not less than 832 micrograms and not more than 970 micrograms of cefonicid per milligram on an anhydrous basis. If the cefonicid sodium is packaged for dispensing, its cefonicid content is not less than 832 micrograms and not more than 970 micrograms of cefonicid per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefonicid that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 5.0 percent.

(v) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 3.5 and not more than 6.5.

(vi) The specific rotation in a methanol solution containing 10 milligrams of cefonicid sodium per milliliter at 25° C is −42°±5°.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefonicid content, sterility, pyrogens, moisture, pH, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug;

(*1*) For all tests except sterility: 10 packages, each containing at least 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefonicid content.* Proceed as directed in § 436.350 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, and a column packed with octadecyl silane bonded silica ranging from 3 to 30 micrometers in particle size. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.2M Ammonium phosphate solution.* Transfer 23.0 grams of ammonium dihydrogen phosphate to a 1-liter volumetric flask. Dissolve and dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix 0.2*M* ammonium phosphate solution : methyl alcohol : distilled water (1 : 2.5 : 16.5). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Prepare the working standard solution fresh before injection by dissolving an accurately weighed portion of the cefonicid working standard with sufficient mobile phase as described in paragraph (b)(1)(i)(*b*) of this section to obtain a solution containing approximately 20 micrograms of cefonicid per milliliter.

(*b*) *Preparation of sample solutions*—(*1*) *Product not packaged for dispensing (micrograms of cefonicid per milligram).* Dissolve an accurately weighed portion of the sample with sufficient mobile phase as described in paragraph (b)(1)(i)(*b*) of this section to obtain a concentration of approximately 20 micrograms of cefonicid per milliliter.

(*2*) *Product packaged for dispensing.* Determine both micrograms of cefonicid per milligram of the sample and milligrams of cefonicid per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(ii)(*b*)(*2*)(*i*) and (*ii*) of this section.

(*i*) *Micrograms of cefonicid per milligram.* Dissolve an accurately weighed portion of the sample with sufficient mobile phase as described in paragraph (b)(1)(i)(*b*) of this section to obtain a concentration of approximately 20 micrograms of cefonicid per milliliter.

(*ii*) *Milligrams of cefonicid per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of the solution thus obtained with sufficient mobile phase to obtain a concentration of approximately 20 micrograms of cefonicid per milliliter.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.3 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates.

(*c*) *Resolution factor.* Prepare a resolution solution containing desacetyl cefonicid by heating a 200-microgram-

per-milliliter solution of cefonicid working standard in mobile phase described in paragraph (b)(1)(i)(*b*) of this section, on a steam bath for 30 minutes. Inject a known volume between 10 and 20 microliters of the desacetyl cefonicid containing solution in the same manner as described for the standard solution. The resolution factor (*R*) between cefonicid and desacetyl cefonicid is satisfactory if it is not less than 1.1.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in § 436.350(b) of this chapter.

(iv) *Calculations*—(*a*) Calculate the micrograms of cefonicid per milligram of sample as follows:

$$\text{Micrograms of cefonicid per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefonicid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefonicid peak in the chromatogram of the cefonicid working standard;

P_s=Cefonicid activity in the cefonicid working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(*b*) Calculate the cefonicid content of the container as follows:

$$\text{Milligrams of cefonicid per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefonicid peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefonicid peak in the chromatogram of the cefonicid working standard;

P_s=Cefonicid activity in the cefonicid working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefonicid per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 50 milligrams per milliliter.

(6) *Specific rotation.* Dissolve and dilute an accurately weighed sample with sufficient methanol to obtain a concentration of approximately 10 milligrams of cefonicid sodium per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(7) *Identity.* The high-performance liquid chromatogram of the sample, determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the cefonicid working standard.

[49 FR 34348, Aug. 30, 1984; 49 FR 44460, Nov. 7, 1984, as amended at 54 FR 41824, Oct. 12, 1989; 55 FR 11583, Mar. 29, 1990]

§ 442.21 Cephaloglycin dihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate is the dihydrate form of 7-(D-α-aminophenylacetamido) cephalosporanic acid. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephaloglycin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture is not less than 8.2 and not more than 12 percent.

(iv) Its pH in an aqueous suspension containing 50 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(v) Its cephaloglycin content is not less than 95 and not more than 104 percent on an anhydrous basis.

(vi) It gives a positive identity test for cephaloglycin dihydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, cephaloglycin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient sterile distilled water to give a stock solution of 100 micrograms of cephaloglycin per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 50 milligrams per milliliter.

(5) *Cephaloglycin content.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the cephaloglycin content as follows:

$$\text{Percent cephaloglycin content} = \frac{(A - B)\ (\text{normality of perchloric acid reagent})\ (405.4)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100 - m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture content of the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19040, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 442.22a Sterile cefmenoxime hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefmenoxime hydrochloride is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-, hydrochloride (2:1), [6*R*-[6α,7β(*Z*)]]-. It is so purified and dried that:

(i) Its cefmenoxime content is not less than 869 and not more than 1,015 micrograms of cefmenoxime per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) It passes the identity test.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefmenoxime content, sterility, pyrogens, moisture, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(B) For sterility testing: 1 package containing approximately 6 grams of a composite sample.

(b) *Tests and methods of assay*—(1) *Cefmenoxime content.* Proceed as directed in § 436.363 of this chapter, using

ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume between 10 and 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Phosphate buffer solution, pH 6.8.* Dissolve 6.4 grams of monobasic potassium phosphate and 18.9 grams of dibasic sodium phosphate in 750 milliliters of water. Adjust the pH to 6.8 with 1*N* sodium hydroxide and dilute to 1,000 milliliters.

(B) *Internal standard solution.* Dissolve and dilute 0.15 gram of phthalimide in methanol to 100 milliliters.

(C) *Mobile phase.* Mix water:acetonitrile:glacial acetic acid (50:10:1). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve approximately 50 milligrams of the cefmenoxime working standard, accurately weighed, in 10 milliliters of 0.1*M* phosphate buffer solution, pH 6.8 and dilute to 50 milliliters with mobile phase. Transfer 4.0 milliliters of this solution to a 50-milliliter volumetric flask, add 20 milliliters of internal standard solution and dilute to volume with mobile phase to obtain a solution containing 80 micrograms of cefmenoxime per milliliter.

(B) *Sample solution.* Dissolve approximately 50 milligrams of cefmenoxime sample, accurately weighed, in 10 milliliters of 0.1*M* phosphate buffer solution, pH 6.8. Dilute to 50 milliliters with mobile phase. Transfer 4.0 milliliters of this solution to a 50-milliliter volumetric flask, add 20 milliliters of internal standard solution and dilute to volume with mobile phase.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) for the cefmenoxime peak is satisfactory if it is not more than 1.6 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,200 theoretical plates for the cefmenoxime peak.

(C) *Resolution.* The resolution (*R*) between the peak for cefmenoxime and phthalimide is satisfactory if it is not less than 2.3.

(D) *Coefficient of variation.* The coefficiednt of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.363(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of cefmenoxime per milligram of sample as follows:

$$\text{Micrograms of cefmenoxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of cefmenoxime peak in the chromatogram of the sample/Area of internal standard peak;

R_s=Area of the cefmenoxime peak in the chromatogram of the cefmenoxime working standard/Area of internal standard peak;

P_s=Cefmenoxime activity in the cefmenoxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except in lieu of diluting fluid A use diluting fluid H.

(3) *Pyrogens.* Proceed as directed in § 436.32(i) of this chapter, using a solution containing 60 milligrams per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section and the titration procedure described in paragraph (e)(3) of that section, except:

(i) In lieu of 3 milliliters of anhydrous methanol solution, inject 20 milliliters of a formamide:methanol solution (2:1) into the container and shake to dissolve the contents (prior to use in preparation of the formamide:methanol solution, dry 500

grams of formamide over 20 grams of anhydrous sodium sulfate for 24 hours);

(ii) Rinse the syringe, needle, and immediate container with two separate 5-milliliter portions of anhydrous methanol, in lieu of one 3-milliliter portion of anhydrous methanol; and

(iii) In §436.201(e)(3) of this chapter, add a sufficient volume of the formamide:methanol solution (2:1) to cover the electrodes in the dry titrating vessel, in lieu of 20 milliliters of solvent A before starting the titration.

(5) *Identity*. Using a 0.0025-percent solution of the sample in 0.1*M* phosphate buffer, pH 6.8 and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cefmenoxime working standard similarly tested.

(6) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[53 FR 13402, Apr. 25, 1988; 53 FR 19368, May 27, 1988]

§442.23a Sterile cephaloridine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cephaloridine is 7-[α-(2-thienyl)-acetamido]-3-(1-pyridyl-methyl)-3-cephem-4-carboxylic acid betaine. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephaloridine per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephaloridine that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 2.5 percent.

(vi) Its pH in an aqueous solution is not less than 3.5 and not more than 6.

(vii) The specific rotation in an aqueous solution containing 10 milligrams of cephaloridine per milliliter at 25° C. is +48°±4°.

(viii) It is crystalline.

(ix) The ultraviolet absorption spectrum between the wavelengths of 220 and 310 nanometers compares qualitatively to that of the cephaloridine working standard. The ratio of the absorbance of the maximum at the wavelength of 240 nanometers to that of the shoulder at 255 nanometers is not less than 1.05 and not more than 1.17.

(2) *Labeling*. It shall be labeled in accordance with the requirements prescribed by §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, specific rotation, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing at least 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 13 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation*. Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay, distilled water for the iodometric assay or hydroxylamine colorimetric assay, to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water as specified above to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephaloridine per milliliter (estimated).

(*b*) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter. If it is packaged for dispensing, dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* HCl, and the assay should be completed within 1 hour after the sample and standard are first put into solution. The working standard should be dried as described in § 436.200(a) of this chapter.

(*c*) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephaloridine per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of cephaloridine per milliliter. If it is packaged for dispensing, however, use the solution obtained after reconstituting the drug as directed in the labeling.

(7) *Specific rotation*. Dilute an accurately weighed sample with sufficient distilled water to give a concentration of approximately 10 milligrams of cephaloridine per milliliter. Proceed as directed in § 436.210 of this chapter using a 2.0-decimeter polarimeter tube.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity*. Using a 0.0025-percent solution of the sample in water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cephaloridine working standard similarly tested.

[39 FR 19040, May 30, 1974, as amended at 43 FR 9800, Mar. 10, 1978; 50 FR 19919, May 13, 1985]

§ 442.25a Sterile cephalothin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile cephalothin sodium is the sodium salt of the compound formed by reaction of thiophene-2-acetic acid with 7-amino-cephalosporanic acid. The 7-amino-cephalosporanic acid is obtained from a kind of cephalosporin. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of cephalothin per milligram on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephalothin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 1.5 percent.

(vi) Its pH in an aqueous solution is not less than 4.5 and not more than 7.0.

(vii) The specific rotation in an aqueous solution containing 50 milligrams of cephalothin sodium per milliliter at 25° C is +129°±5°.

(viii) It gives a positive identity test.

(ix) It is crystalline.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing and is intended for both intravenous and intramuscular use, each vial shall contain the equivalent of 1 gram of cephalothin; except that if it is packaged for dispensing and is intended solely for intravenous use, each vial shall contain the equivalent of 4 grams of cephalothin.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, if it is packaged for dispensing, each package shall bear on its label and labeling, the following statement: ''After reconstitution, store in a

refrigerator and use within 48 hours. If kept at room temperature, use within 6 hours.''

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of test and assay on the batch for potency, sterility, pyrogens, loss on drying, pH, specific rotation, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing equal portions of approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay, distilled water for the iodometric assay or hydroxylamine colorimetric assay, to give a stock solution of convenient concentration; also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water as specified above to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephalothin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter. If it is packaged for dispensing, dilute an aliquot of the stock solution with distilled water to the prescribed concentration.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* HCl, and the assay should be completed within 1 hour after the sample and standard are first put into solution. The working standard should be dried as described in § 436.200(a) of this chapter.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephalothin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams per milliliter; however, if it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(7) *Specific rotation.* Dilute an accurately weighed sample with sufficient distilled water to give a concentration of approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Using a 0.0025-percent solution of the sample in water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the

cephalothin working standard similarly tested.

[39 FR 19040, May 30, 1974, as amended at 46 FR 46312, Sept. 18, 1981; 48 FR 11427, Mar. 18, 1983; 50 FR 19919, May 13, 1985]

§ 442.27 Cephalexin monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate is the monohydrate form of 7-(D-*alpha*-amino-*alpha*- phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of cephalexin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.0 nor more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 3.0 nor more than 5.5.

(v) When calculated on an anhydrous basis, its absorptivity at 262 nanometers is not less than 95 percent and not more than 104 percent of that of the cephalexin standard similarly treated and corrected for potency.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 20 micrograms of cephaxelin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 50 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve accurately weighed portions of approximately 50 milligrams each of the sample and standard in 250 milliliters of distilled water. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with distilled water. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of each solution at 262 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where:

m=percent moisture in the sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5

percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity*. Proceed as directed in §436.203 of this chapter.

[39 FR 19040, May 30, 1974, as amended at 50 FR 19919, May 13, 1985; 52 FR 35912, Sept. 24, 1987]

§442.28 Cephalexin hydrochloride monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cephalexin hydrochloride monohydrate is the hydrochloride salt of 7-*(D-alpha-* amino-*alpha-*phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid monohydrate. It is so purified and dried that:

(i) Its potency is not less than 800 micrograms and not more than 880 micrograms of cephalexin per milligram on an ''as is'' basis.

(ii) Its moisture content is not less than 3.0 nor more than 6.5 percent.

(iii) The pH of an aqueous solution containing 10 milligrams per milliliter is not less than 1.5 nor more than 3.0.

(iv) It gives a positive identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cephalexin potency, moisture, pH, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Cephalexin potency*. Proceed as directed in §442.40(b)(1)(ii), except that ''cephalexin'' is substituted at each occurrence of ''cephradine''.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(4) *Identity*. Proceed as directed in §436.367 of this chapter.

(5) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[54 FR 48860, Nov. 28, 1989]

§442.29a Sterile cephapirin sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cephapirin sodium is the sodium salt of 7-[α(4-pyridylthio) -acetamido]-cephalosporanic acid. It is a white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 855 micrograms and not more than 1,000 micrograms of cephapirin per milligram on an ''as is'' basis. If it is packaged for dispensing, its content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cephapirin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams of cephapirin per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its cephapirin content is not less than 92 percent and not more than 105 percent on an anhydrous basis.

(viii) It gives a positive identity test for sodium cephapirin.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, cephapirin content, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 9 packages, each containing approximately 500 milligrams, and 1 package containing approximately 5 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 14 immediate containers, except if each contains less than 1 gram, a minimum of 19 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single–dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephapirin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in § 436.204 of this chapter. In addition if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to the prescribed concentration.

(iii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 436.205 of this chapter. In addition, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with distilled water to the prescribed concentration.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 100 milligrams of cephapirin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Cephapirin content*. Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the cephapirin content as follows:

$$\text{Percent cephapirin content} = \frac{(A - B)\ (\text{normality of perchloric acid reagent})\ (222.7)\ (100)\ (100)}{(\text{Weight of sample in milligrams})\ (100 - m)}$$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample.

B=Milliliters of perchloric acid reagent used in titrating the blank.

m=Percent moisture content of the sample.

(8) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared

as directed in paragraph (b)(1) of that section.

(9) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19040, May 30, 1974, as amended at 40 FR 23725, June 2, 1975; 50 FR 19919, May 13, 1985]

§ 442.40 Cephradine.

(a) *Requirements of certification*—(1) *Standards of identity, strength, quality, and purity*. Cephradine is (6*R*, 7*R*)-7-[(*R*)-2-amino-2-(1,4-cyclohexadien-1-yl) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of cephradine per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 6.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its cephalexin content is not more than 5 percent on an anhydrous basis.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram of cephradine per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cephradine*—(*a*) *Typical equipment*. Use automated equipment capable of performing the following functions: Introduction of sample into reaction vessels, addition of reagents to the samples to form reaction mixtures, incubation of the reaction mixtures, colorimetric determination of the reaction product at 480 nanometers using a 1-centimeter tubular flow cuvette, and documentation of the results with a strip chart recorder. A suitable system is the Auto Analyzer II equipment consisting of a Solid or Liquid Sampler II, a twenty channel Pump III, a colorimeter equipped with a 1-centimeter tubular flow cuvette and light filters producing incident light at 480 nanometers, and a strip chart recorder with scale expander.

(*b*) *Reagents*—(*1*) *Hydroxylamine hydrochloride solution*. Dissolve 20 grams of hydroxylamine hydrochloride and 5 milliliters of emulsifying stock solution (prepared to contain 100 milligrams of polyoxyethylene fatty alcohol ether, such as Brij-35 or equivalent, per 100 milliliters distilled water) in sufficient distilled water to make 1 liter.

(*2*) *Buffer*. Dissolve 173 grams of sodium hydroxide and 20.6 grams of sodium acetate in sufficient distilled water to make 1 liter. Dilute 75 milliliters of this solution with distilled water to 500 milliliters.

(*3*) *3.3N Sulfuric acid*. Dilute 91 milliliters of concentrated sulfuric acid to 1 liter with distilled water.

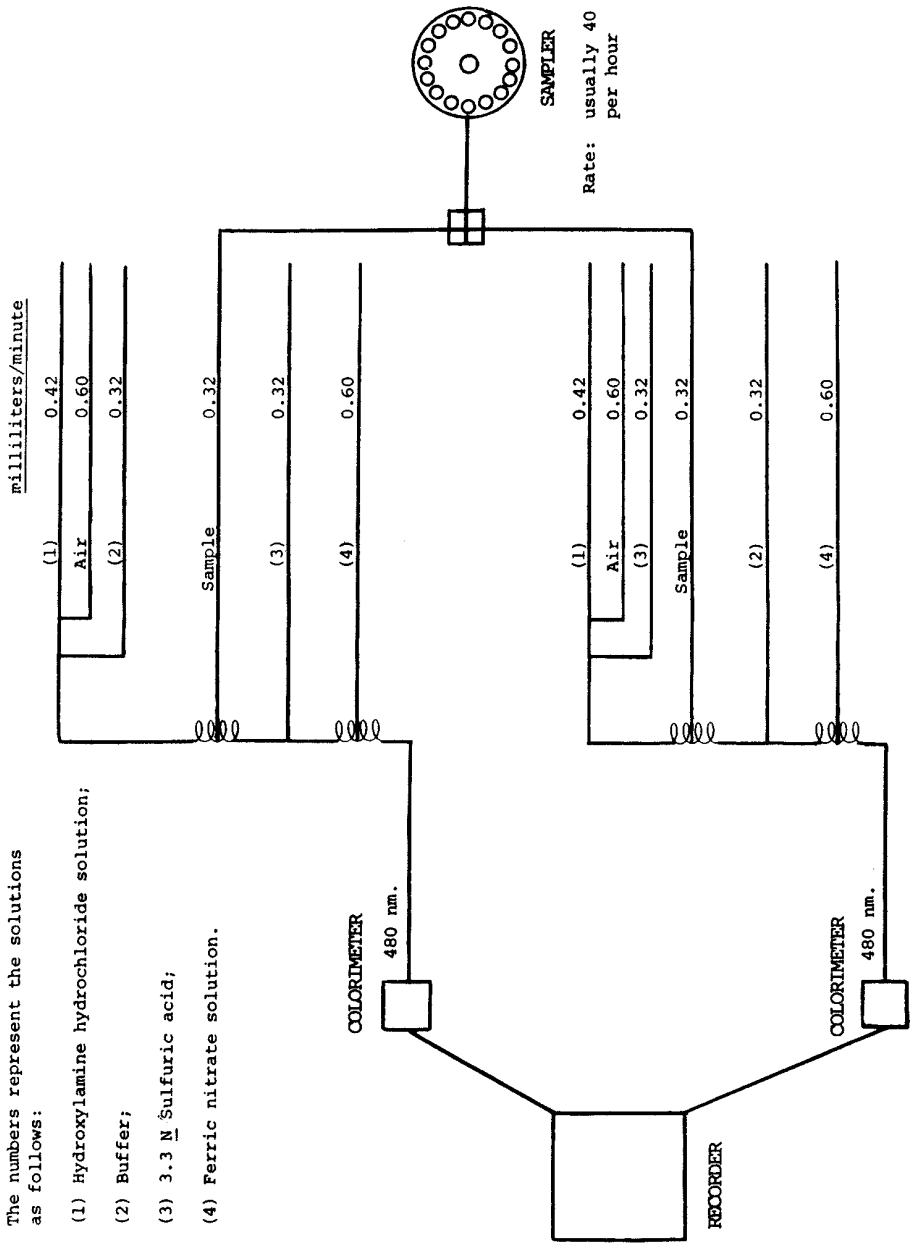
(*4*) *Ferric nitrate solution*. Dissolve 300 grams of ferric nitrate nonahydrate ($9H_2O$) in a mixture of 2.8 milliliters of concentrated sulfuric acid and sufficient distilled water to make 1 liter.

(*c*) *Preparation of working standard solutions*. Dissolve and dilute an accurately weighed portion of the cephradine working standard in sufficient distilled water to obtain a concentration of 1 milligram of cephradine per milliliter.

(*d*) *Preparation of sample solutions*. Dissolve an accurately weighed portion of the sample in distilled water and

further dilute to 1 milligram of cephradine per milliliter (estimated).

(*e*) *Procedure.* Use the standard and sample solutions prepared as indicated in paragraph (b)(1)(ii) (*c*) and (*d*) of this section respectively. The arrangement of the apparatus and flow of samples and reagents are shown in the manifold diagram set forth in this paragraph (b)(1)(ii)(*e*). The sampler rate is usually 40 per hour, but may be varied.



(*f*) Calculate the potency of the sample in micrograms per milligram as follows:

$$\text{Micrograms of cephradine per milligram of sample} = \frac{A_u \times P_s \times 100}{A_s \times W_u \times (100 - m)}$$

where:

A_u=Absorbance of sample solution;

P_s=Potency of working standard solution in micrograms, per milliliter;

A_s=Absorbance of working standard solution;

W_u=Milligrams of sample per milliliter of sample solution;

m=Percent moisture in sample.

(iii) *High-pressure liquid chromatographic assay.* Proceed as directed in § 436.337 of this chapter, preparing the sample as described in paragraph (e)(3)(i) of that section.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Cephalexin content.* Proceed as directed in § 436.337 of this chapter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 1 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[40 FR 26270, June 23, 1975, as amended at 45 FR 16474, Mar. 14, 1980; 46 FR 25608, May 8, 1981; 48 FR 51293, Nov. 8, 1983; 49 FR 47485, Dec. 5, 1984; 50 FR 19919, May 13, 1985]

§ 442.40a Sterile cephradine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine is 7-[D-2 - amino -2- (1,4 - cyclohexadien - 1 -yl) acetamido] - 3 - methyl - 8 - oxo - 5-thia - 1- azabicyclo[4.2.0]oct -2 - ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 and not more than 1,050 micrograms of cephradine per milligram on the anhydrous basis. If it is packaged for dispensing, its cephradine content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephradine that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 6.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(vii) Its cephalexin content is not more than 5 percent on an anhydrous basis.

(viii) It passes the identity test.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, cephalexin content, identity, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for manufacturing use:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 1 package containing approximately 6 grams of a composite sample.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution containing 1.0 milligram of cephradine per milliliter (estimated); also, if it is packaged for dispensing, reconstitute the sample as directed in the labeling, except use distilled water in lieu of reconstituting fluid. Then using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container. Dilute with solution 1 to give a

stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in §442.40(b)(1)(ii). If packaged for dispensing, reconstitute the sample as directed in the labeling using distilled water instead of the reconstituting fluid. Further dilute an aliquot of this solution with distilled water to 1 milligram of cephradine per milliliter (estimated).

(iii) *High-pressure liquid chromatographic assay.* Proceed as directed in §436.337 of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(g) of this chapter, using a solution containing 80 milligrams of cephradine per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in §436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(7) *Cephalexin content.* Proceed as directed in §442.40(b)(5).

(8) *Identity.* Proceed as directed in §436.211 of this chapter, using the 1 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(9) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[40 FR 51626, Nov. 6, 1975, as amended at 43 FR 14646, Apr. 7, 1978; 49 FR 47485, Dec. 5, 1984; 50 FR 19919, May 13, 1985]

§442.41 Cephradine dihydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine dihydrate is the dihydrate form of (6*R*,7*R*)-7-[(*R*)-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,050 micrograms of cephradine per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 8.5 percent and not more than 10.5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 6.0.

(v) Its cephalexin content is not more than 5 percent on an anhydrous basis.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cephradine.* Proceed as directed in §442.40(b)(1)(ii).

(iii) *High-pressure liquid chromatographic assay.* Proceed as directed in §436.337 of this chapter, preparing the sample as described in paragraph (e)(3)(i) of that section.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of thichapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Cephalexin content.* Proceed as directed in § 436.337 of this chapter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 1 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[47 FR 11856, Mar. 19, 1982, as amended at 49 FR 47485, Dec. 5, 1984; 50 FR 19919, May 13, 1985]

§ 442.50a Sterile ceforanide.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceforanide is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[2-(amino-methyl)phenyl]acetyl]amino]-3-[[[1-(carboxymethyl)-1*H*-tetrazol-5-yl]-thio]methyl]-8-oxo-,(6*R-trans*)-. It is a white to off-white powder. It is so purified and dried that:

(i) Its ceforanide content is not less than 900 micrograms and not more than 1,050 micrograms of ceforanide per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 5.0 percent.

(v) Its pH in an aqueous suspension containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(vi) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for ceforanide content, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: One package containing approximately 6 grams of a composite sample.

(b) *Tests and methods of assay*—(1) *Ceforanide content.* Proceed as directed in § 436.348 of this chapter, preparing the sample and calculating the ceforanide content as follows:

(i) *Preparation of sample solution.* Prepare a solution containing 1.0 milligram per milliliter in mobile phase. Inject each sample within 5 minutes after dissolution.

(ii) *Calculations.* Calculate the micrograms of ceforanide per milligram of sample as follows:

$$\begin{matrix}\text{Micrograms of}\\ \text{ceforanide}\\ \text{per milligram}\end{matrix} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u = Area of the ceforanide peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the ceforanide peak in the chromatogram of the ceforanide working standard;

P_s = Ceforanide activity in the ceforanide working standard solution in micrograms per milliliter; and

C_u = Milligrams of sample per milliliter of sample solution.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except:

(i) In paragraph (e)(1)(i)(*a*) of that section, use diluting fluid G in lieu of diluting fluid A; and

(ii) In lieu of three 100-milliliter quantities of diluting fluid A in paragraph (e)(2) of that section, filter three 100-milliliter quantities of diluting fluid D followed by a 100-milliliter quantity of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, except suspend 1 gram of sterile ceforanide in 12.5 milliliters of pyrogen-free water (diluent 1). Add 320 milligrams of pyrogen-free *L*-lysine base, shake to dissolve the mixture. If the mixture is not dissolved, add an amount of *L*-lysine necessary to obtain a solution. The test sample should contain not more than a total of 340 milligrams of *L*-lysine. Dilute the resulting solution to 20 milliliters. Use a test dose of 1 milliliter of the 50 milligrams per milliliter test solution per kilogram of rabbit weight.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 50 milligrams per milliliter.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation as described in paragraph (b)(2) of that section.

[49 FR 25847, June 25, 1984; 49 FR 40006, Oct. 12, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.52 Cefotetan.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefotetan is (6*R*,7*S*)-4-[[2-carboxy-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-7-yl]-carbamoyl]-1,3-dithietane-$\Delta^{2,\alpha}$-malonamic acid. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,030 micrograms of cefotetan activity per milligram on the anhydrous basis.

(ii) Its moisture content is not more than 2.5 percent.

(iii) It gives a positive identity test for cefotetan.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.216 of this chapter, except use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not exceeding 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Diluting solution*. Mix water:methanol:acetonitrile (90:5:5).

(B) *Mobile phase*. Mix 0.1*M* phosphoric acid:glacial acetic acid:methanol:acetonitrile (1700:100:105:105). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution*. Accurately weigh approximately 50 milligrams of the cefotetan working standard into a 250-milliliter volumetric flask containing 12.5 milliliters of methanol. Swirl the flask for several minutes, then add 12.5 milliliters of acetonitrile. Swirl the flask until the cefotetan is dissolved. Dilute to volume with water to obtain a solution containing approximately 200 micrograms of cefotetan per milliliter. Mix well. Protect the working standard solution from light.

(B) *Sample solution*. Dissolve an accurately weighed portion of the sample with sufficient diluting solution described in paragraph (b)(1)(i)(A) of this section to obtain a concentration of approximately 200 micrograms of cefotetan per milliliter.

(C) *Resolution test solution*. Place 10 milliliters of the working standard solution in a stoppered flask containing a few milligrams of magnesium carbonate. Close the flask and sonicate for 10 minutes. If the solution is not slightly turbid, add more magnesium carbonate and repeat sonication. Filter the turbid solution through a 0.5-micron filter and use within 2 hours. As this solution stands, the tautomer concentration increases.

(iii) *System suitability requirements*—(A) *Tailing factor*. The tailing factor (*T*) is satisfactory if it is not more than 1.3 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates.

(C) *Resolution*. The resolution (*R*) between the peak for cefotetan and its tautomer is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation*. The coefficient of variation (S_r in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216 (b) of this chapter. Alternate chromatographic conditions are acceptable provided comparable system suitability requirements are met. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculation*. Calculate the micrograms of cefotetan per milligram of sample as follows:

$$\text{Micrograms of cefotetan per milligram} = \frac{A_U \times P_S \times V_f \times 1{,}000}{A_S \times V_s}$$

where:

A_U = Area of the cefotetan peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the cefotetan peak in the chromatogram of the cefotetan working standard;

P_S = Cefotetan activity in the cefotetan working standard solution in micrograms per milliliter;

V_f = Volume of flask used to dilute standard; and

V_s = Volume of sample diluted.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *Identity*. Proceed as directed in § 436.211 of this chapter using the potassium bromide discs prepared as described in § 436.211(b)(1) of this chapter or the mineral oil mull prepared as described in § 436.211(b)(2) of this chapter.

[59 FR 26940, May 25, 1994, as amended at 60 FR 33712, June 29, 1995]

§ 442.53a Sterile cefotetan disodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cefotetan disodium is a white to off-white lyophilized powder. It is so purified and dried that:

(i) If the cefotetan disodium is not packaged for dispensing, its potency is not less than 830 micrograms and not more than 970 micrograms of cefotetan per milligram on the anhydrous basis. If the cefotetan disodium is packaged for dispensing, its potency is not less than 830 micrograms and not more than 970 micrograms of cefotetan per milligram on the anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefotetan that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) Its pH in an aqueous solution containing 100 milligrams of cefotetan disodium per milliliter is not less than 4.0 and not more than 6.5.

(vi) It gives a positive identity test for cefotetan.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.216 of this chapter, except use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 254 nanometers, a column

packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not exceeding 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Diluting solution.* Mix water:methanol:acetonitrile (90:5:5).

(*b*) *Mobile phase.* Mix 0.1*M* phosphoric acid:glacial acetic acid:methanol:acetonitrile (1700:100:105:105). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Accurately weigh approximately 50 milligrams of the cefotetan working standard into a 250-milliliter volumetric flask containing 12.5 milliliters of methanol. Swirl the flask for several minutes, then add 12.5 milliliters of acetonitrile. Swirl the flask until the cefotetan is dissolved. Dilute to volume with water to obtain a solution containing approximately 200 micrograms of cefotetan per milliliter. Mix well. Protect the working standard solution from light.

(*b*) *Sample solutions*—(*1*) *Product not packaged for dispensing (micrograms of cefotetan per milligram).* Dissolve an accurately weighed portion of the sample with sufficient diluting solution described in paragraph (b)(1)(i)(*a*) of this section, to obtain a concentration of approximately 200 micrograms of cefotetan per milliliter.

(*2*) *Product packaged for dispensing.* Determine both micrograms of cefotetan per milligram of the sample and milligrams of cefotetan per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(ii)(*b*)(*2*)(*i*) and (*ii*) of this section.

(*i*) *Micrograms of cefotetan per milligram.* Dissolve an accurately weighed portion of the sample with sufficient diluting solution described in paragraph (b)(1)(i)(*a*) of this section, to obtain a concentration of approximately 200 micrograms of cefotetan per milliliter.

(*ii*) *Milligrams of cefotetan per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of the solution thus obtained with sufficient diluting solution described in paragraph (b)(1)(i)(*a*) of this section, to obtain a concentration of approximately 200 micrograms of cefotetan per milliliter.

(*c*) *Resolution test solution.* Place 10 milliliters of the working standard solution in a stoppered flask containing a few milligrams of magnesium carbonate. Close the flask and sonicate for 10 minutes. If the solution is not slightly turbid, add more magnesium carbonate and repeat sonication. Filter the turbid solution through a 0.5-micron filter and use within 2 hours. As this solution stands, the tautomer concentration increases.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.3 at 10 percent of peak height in lieu of 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,500 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between the peak for cefotetan and its tautomer is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided comparable system suitability requirements are met. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—(*a*) Calculate the micrograms of cefotetan per milligram of sample as follows:

$$\text{Micrograms of cefotetan per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefotetan peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotetan peak in the chromatogram of the cefotetan working standard;

P_s=Cefotetan activity in the cefotetan working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(*b*) Calculate the cefotetan content of the container as follows:

$$\text{Milligrams of cefotetan per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefotetan peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotetan peak in the chromatogram of the cefotetan working standard;

P_s=Cefotetan activity in the cefotetan working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefotetan per milliliter.

(4) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of cefotetan disodium per milliliter.

(6) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the cefotetan working standard.

[51 FR 20263, June 4, 1986, as amended at 52 FR 35912, Sept. 24, 1987; 55 FR 11583, Mar. 29, 1990]

§ 442.54 Cefpodoxime proxetil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefpodoxime proxetil is (±)-1-hydroxyethyl(+)-(6*R*,7*R*)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate,7²-(*Z*)-(*O*-methyloxime), isopropyl carbonate (ester). It is so purified and dried that:

(i) Its potency is not less than 690 micrograms and not more than 804 micrograms of cefpodoxime activity per milligram, on an anhydrous basis.

(ii) The ratio of its R-epimer to total cefpodoxime is not less than 0.5 and not more than 0.6.

(iii) Its moisture content is not more than 3 percent.

(iv) It gives a positive identity test.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefpodoxime potency, isomer ratio, moisture, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.216 of this chapter, using a suitable thermostatted column heating mechanism to maintain a column temperature of 40 °C, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 15 centimeter X 4.6 millimeter (i.d.) column packed with microparticulate (5 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded to silicas, a flow rate of 0.8 milliliter per minute, and a known injection volume of 2 microliters. The retention time for

the S-epimer is approximately 22 minutes and the retention time for R-epimer is approximately 28 minutes. The internal standard (propylparaben) has a retention time of 34 minutes. Mobile phase, dilution solvent, resolution solution, internal standard solution, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* The mobile phase consists of 420 milliliters of methanol, 580 milliliters of deionized water, and 230 milligrams of *L*-histidine hydrochloride. The pH is adjusted to 2.5±0.1 using 2*N* sulfuric acid. The mobile phase must be at room temperature for a correct pH measurement. The methanol concentration may be adjusted to achieve comparable retention times from column to column. Increasing methanol reduces retention times. Filter the mobile phase through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just before its introduction into the chromatograph.

(ii) *Dilution solvent.* Prepare a solvent for dilution by thoroughly mixing 495 milliliters of deionized water, 495 milliliters of acetonitrile, and 10 milliliters of acetic acid in an appropriate container.

(iii) *Resolution solution.* Prepare a 1 milligram per milliliter solution of any bulk containing ANTI–A in dilution solvent. Use this solution to determine the resolution between ANTI–A and the later-eluting drug epimer (R-epimer). Alternately, the resolution factor can be determined between the R and S isomers.

(iv) *Internal standard solution.* Prepare a solution of propylparaben in dilution solvent at a concentration of 10 milligrams per milliliter.

(v) *Preparation of working standard solutions.* Accurately weigh approximately 42 milligrams of the cefpodoxime proxetil working reference standard add 3 milliliters of internal standard solution and 25 milliliters of dilution solvent. The standard solution is stable for at least 48 hours. Refrigeration is not recommended.

(vi) *Sample solution.* Accurately weigh approximately 42 milligrams of the sample, add 3 milliliters of internal standard and 25 milliliters of dilution solvent. The sample solution is stable for at least 48 hours. Refrigeration is not recommended.

(vii) *System suitability requirements*—(A) *Asymmetry factor.*The asymmetry factor (A_s) is satisfactory if it is not less than 0.8 and not more than 1.1 for the R-epimer of cefpodoxime peak.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 5 for the R-epimer peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for ANTI–A and the peak for the R-epimer is satisfactory if it is not less than 1.3. Alternately, the resolution factor (R) between the peak for the R-epimer and the peak for the S-epimer of cefpodoxime is not less than 11.

(D) *Coefficient of variation (Relative standard deviation).* The coefficient of variation ($S_R$in percent of 5 replicate injections) is satisfactory if it is not more than 2 percent.

(E) *Capacity factor (k').* The capacity factor (k') for the R-epimer of cefpodoxime is satisfactory if it is not less than 10.4 and not more than 15.6.

(F) If the system suitability parameters in this paragraph (b)(1)(iv) have been met, then proceed as described in § 436.216(b) of this chapter.

(viii) *Calculations.* Calculate the micrograms of cefpodoxime proxetil per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of cefpodoxime proxetil per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u = Ratio of cefpodoxime proxetil peaks area (sum of both epimers) to the internal standard peak response in the sample solution;

R_s = Ratio of cefpodoxime proxetil peaks

area (sum of both epimers) to the internal standard peak response in the working standard solution;

P_s = Cefpodoxime proxetil activity of the working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Isomer ratio.* Using the procedure described in paragraph (b)(1) of this section, calculate the ratio of the R-epimer (Ab) to the sum of the S-epimer and R-epimer (Aa and Ab), by the equation

Isomer Ratio = Ab/(Aa + Ab)

where:

Aa = Area of the early eluting S-epimer peak; and

Ab = Area of the late eluting R-epimer peak.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter, except use 30 milliliters of solvent C instead of 20 milliliters of solvent A.

(4) *Identity.* Proceed as directed in § 436.211 of this chapter, using the mineral oil mull prepared as described in paragraph (b)(2) of that section.

[60 FR 58231, Nov. 27, 1995]

§ 442.55 Ceftriaxone sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftriaxone sodium is the 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-,disodium salt, [6*R*-[6*alpha*, 7*beta*(*Z*)]]-. It is so purified and dried that:

(i) Its ceftriaxone potency is not less than 795 micrograms of ceftriaxone per milligram on an anhydrous free acid basis.

(ii) Its moisture content is not less than 8 percent and not more than 11 percent.

(iii) The pH of an aqueous solution containing the equivalent of 100.0 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(iv) It is crystalline.

(v) It gives a positive identity test for ceftriaxone.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for ceftriaxone potency, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Ceftriaxone potency.* Proceed as directed in § 442.55a(b)(1) of this chapter, except prepare the sample solution and calculate the micrograms of ceftriaxone free acid per milligram as follows:

(i) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample with sufficient water to obtain a concentration of 180 micrograms of ceftriaxone activity per milliliter. Prepare the sample solution just prior to its introduction into the chromatograph.

(ii) *Calculation.* Calculate the micrograms of ceftriaxone anhydrous free acid per milligram as follows:

$$\text{Micrograms of ceftriaxone anhydrous free acid per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the ceftriaxone peak in the chromatogam of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftriaxone peak in the chromatogram of the ceftriaxone working standard;

P_s=Ceftriaxone activity in the ceftriaxone working standard solution in micrograms of anhydrous free acid per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using a potassium bromide disc containing 1.3 milligrams of ceftriaxone sodium in 300 milligrams of potassium bromide, prepared as described in paragraph (b)(1) of that section.

[52 FR 44860, Nov. 23, 1987, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.55a Sterile ceftriaxone sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftriaxone sodium is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-, disodium salt, [6*R*-[6α,7β(*Z*)]]-. It is so purified and dried that:

(i) If the ceftriaxone sodium is not packaged for dispensing, its ceftriaxone potency is not less than 795 micrograms of ceftriaxone per milligram on an anhydrous free acid basis. If the ceftriaxone sodium is packaged for dispensing, its ceftriaxone potency is not less than 776 micrograms of ceftriaxone per milligram on an anhydrous free acid basis and also, each container contains not less than 90 percent and not more than 115 percent of the number of milligrams of ceftriaxone that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not less than 8 percent and not more than 11 percent.

(v) Its pH in an aqueous solution containing the equivalent of 100.0 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(vi) It is crystalline.

(vii) It gives a positive identity test for ceftriaxone.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for ceftriaxone potency, and if packaged for dispensing, potency and container content, sterility, pyrogens, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for manufacturing use:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ceftriaxone potency and container content.* Proceed as directed in § 436.354 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 270 nonometers (or 254 nanometers fixed mercury source), and a column packed with a five-micron octadecyl reverse phase packing or equivalent; and also, using the following system suitability requirements, reagents, working standard, test and sample solutions, and calculations:

(i) *System suitability requirements*—(*a*) *Capacity factor.* The capacity factor (k) for the ceftriaxone peak is satisfactory if it is not less than 2 and not more than 5.

(*b*) *Resolution.* The resolution (R) between the peak for ceftriaxone E-isomer and ceftriaxone is satisfactory if it is not less than 3.0.

(*c*) *Asymmetry factor.* The asymmetry factor (A_s) is satisfactory if it is not more than 1.6 at 10 percent of the peak height.

(*d*) *Efficiency of the column.* The efficiency of the column (h_r) is satisfactory if it is less than 20 (equivalent to a value of 1,500 or greater theoretical plates when using a 15-centimeter column with 5-micrometer-size particles).

(*e*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is less than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in § 436.354(b) of this chapter.

(ii) *Reagents*—(*a*) *pH 7.0 phosphate buffer.* Dissolve 13.6 grams of dibasic potassium phosphate and 4.0 grams of monobasic potassium phosphate in sufficient water to make 1,000 milliliters. Adjust to pH 7.0 ±0.1 with 18*N* phosphoric acid or 10*N* potassium hydroxide.

(*b*) *pH 5.0 citrate buffer.* Dissolve 25.8 grams of sodium citrate in 500 milliliters of water. Adjust the pH to 5.0±0.1 with 20 percent aqueous citric acid, and dilute to 1,000 milliliters with water.

(*c*) *Mobile phase.* Dissolve 4.0 grams of tetraheptylammonium bromide with 500 milliliters of acetonitrile. Add 440 milliliters of water, 55 milliliters of pH 7.0 phosphate buffer, and 5 milliliters of pH 5.0 citrate buffer. Mix and dilute 800 milliliters of this solution with 200 milliliters of distilled water. Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(iii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the ceftriaxone working standard with sufficient water to obtain a solution containing approximately 180 micrograms of ceftriaxone activity per milliliter. Prepare the working standard solution just prior to its introduction into the chromatograph.

(*b*) *Preparation of test solution.* Dissolve together accurately weighed portions of the ceftriaxone working standard and the ceftriaxone sodium E-isomer reference standard with sufficient water to obtain a solution containing approximately 160 micrograms of ceftriaxone activity per milliliter of each standard. Prepare the test solution just prior to its introduction into the chromatograph.

(*c*) *Preparation of sample solution.* Prepare the sample solution just prior to its introduction into the chromatograph.

(*1*) *Product not packaged for dispensing (micrograms of ceftriaxone anhydrous free acid per milligram*). Dissolve an accurately weighed portion of the sample with sufficient water to obtain a concentration of 180 micrograms of ceftriaxone activity per milliliter.

(*2*) *Product packaged for dispensing.* Determine both potency (micrograms of ceftriaxone anhydrous free acid per milligram of the sample) and container content (milligrams of anhydrous free acid ceftriaxone per container). Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(iii)(*b*)(*2*) (*i*) and (*ii*) of this section.

(*i*) *Micrograms of ceftriaxone anhydrous free acid per milligram.* Dissolve an accurately weighed portion of the sample with sufficient water to obtain a concentration of approximately 180 micrograms of ceftriaxone activity per milliliter.

(*ii*) *Milligrams of ceftriaxone per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the potency contained in a given volume of the resulting preparation, remove an accurately measured representative portion from each container. Dilute the aliquot of the solution thus obtained with sufficient water to obtain a concentration of approximately 180 micrograms of ceftriaxone activity per milliliter.

(iv) *Calculations.* (*a*) Calculate the micrograms of ceftriaxone anhydrous free acid per milligram as follows:

$$\begin{array}{c}\text{Micrograms of}\\ \text{ceftriaxone anhydrous}\\ \text{free acid per milligram}\end{array} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the ceftriaxone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftriaxone peak in the chromatogram of the ceftriaxone working standard;

P_s=Ceftriaxone activity in the ceftriaxone working standard solution in micrograms of anhydrous free acid per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

(*b*) Calculate the ceftriaxone anhydrous free acid content of the container as follows:

$$\text{Milligrams of ceftriaxone anhydrous free acid per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ceftriaxone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftriaxone peak in the chromatogram of the ceftriaxone working standard;

P_s=Ceftriaxone activity in the ceftriaxone working standard solution in micrograms of anhydrous free acid per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(h) of this chapter, using a solution containing 20 milligrams of ceftriaxone per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a potassium bromide disc containing 1.3 milligrams of ceftriaxone sodium in 300 milligrams of potassium bromide, prepared as described in paragraph (b)(1) of that section.

[50 FR 9999, Mar. 13, 1985; 50 FR 11690, Mar. 25, 1985; 50 FR 18243, Apr. 30, 1985, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.58a Sterile cefotiam dihydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotiam dihydrochloride is 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[(2-amino-4-thiazolyl)acetyl]-amino-3[[[1-[2-(dimethylamino)ethyl]-1*H*-tetrazol-5-yl]-thio]methyl]-8-oxo-, dihydrochloride, (6*R*-trans)-. It is so purified and dried that:

(i) Its potency is not less than 790 and not more than 925 micrograms of cefotiam per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 7.0 percent.

(v) It passes the identity test.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(B) For sterility testing: One package containing approximately 6 grams of a composite sample.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Dissolve 13.1 grams of ammonium sulfate in 850 milliliters of water. Adjust the pH to 6.5 with dilute aqueous ammonia. Add 150 milliliters of acetonitrile. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A)

Working standard solution. Dissolve approximately 100 milligrams of the cefotiam working standard, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter.

(B) *Sample solution.* Dissolve approximately 100 milligrams of the sample, accurately weighed, in water and dilute to 100 milliliters. Further dilute with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter (estimated).

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefotiam working standard in water to obtain a solution containing approximately 1.0 milligram of cefotiam activity per milliliter. Heat this solution at 95 °C for 15 minutes. This procedure allows cefotiam lactone to be produced. Dilute 1.0 milliliter of this solution to 100 milliliters with mobile phase.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) for the cefotiam peak is satisfactory if it is not more than 1.76 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1985 theoretical plates for the cefotiam peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for cefotiam and the peak for cefotiam lactone (generated in situ) is satisfactory if it is not less than 4.0.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of cefotiam per milligram of sample as follows:

$$\text{Micrograms of cefotiam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefotiam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotiam peak in the chromatogram of the cefotiam working standard;

P_s=Cefotiam activity in the cefotiam working standard solution in micrograms per milliliter;

C_u=Milligrams of the sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(g) of this chapter, using a solution containing 40 milligrams per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section and the titration procedure described in paragraph (e)(3) of that section, except:

(i) In lieu of 3 milliliters of anhydrous methanol solution, inject 20 milliliters of a formamide:methanol solution (2:1) into the container and shake to dissolve the contents (prior to use in preparation of the formamide:methanol solution, dry 500 grams of formamide over 20 grams of anhydrous sodium sulfate for 24 hours);

(ii) Rinse the syringe, needle, and immediate container with two separate 5-milliliter portions of anhydrous methanol, in lieu of one 3-milliliter portion of anhydrous methanol; and

(iii) In paragraph (e)(3) of that section, add a sufficient volume of the formamide:methanol solution (2:1) to cover the electrodes in the dry titrating vessel, in lieu of 20 milliliters of solvent A before starting the titration.

(5) *Identity.* Using a solution containing 20 micrograms per milliliter of water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the cefotiam working standard similarly tested.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[54 FR 20785, May 15, 1989]

§ 442.60 Cefpiramide.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefpiramide is (6*R*, 7*R*)-7-

[(*R*)-2-(4-hydroxy-6-methyl-nicotinamido)-2-(*p*-hydroxyphenyl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 974 micrograms of cefpiramide activity per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 9.0 percent.

(iii) Its pH in an aqueous suspension containing 5 milligrams per milliliter is not less than 3.0 and not more than 5.0.

(iv) Its total related substances content by high performance liquid chromatography is not more than 2.0 percent. No individual impurity is more than 0.7 percent.

(v) The specific rotation in dimethylformamide solution containing 10 milligrams of cefpiramide per milliliter is −106 ± 6 °C calculated on an anhydrous basis.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, total related substances, specific rotation, identity, and crystallinity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating to a wavelength of 254 nanometers, a 15- to 30-centimeter X 4-millimeter (inside diameter) column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase packing material such as octylsilane bonded to silica, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.01M phosphate buffer.* Dissolve 1.36 grams of monobasic potassium phosphate in 900 milliliters of water. Adjust the pH to 6.8 with 1*N* sodium hydroxide and dilute to 1,000 milliliters with water.

(B) *Mobile phase.* Mix 0.01*M* phosphate buffer: acetonitrile: tetrahydrofuran: methanol (880:40:40:40). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve and dilute an accurately weighed portion of the cefpiramide working standard in sufficient mobile phase to obtain a solution containing 0.25 milligram of cefpiramide activity per milliliter.

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample in mobile phase and further dilute to 0.25 milligram of cefpiramide per milliliter (estimated).

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefpiramide working standard in 0.01*N* sodium hydroxide to obtain a solution containing approximately 1.0 milligram of cefpiramide activity per milliliter. Heat this solution at 95 °C for 10 minutes. This procedure allows cefpiramide lactone to be produced. Dilute 1.0 milliliter of this solution to 20 milliliters with mobile phase.

(iii) *System suitability requirements*)—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 0.95 and not more than 1.4.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of

this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:
L=Length of the column in centimeters;
n=Number of theoretical plates; and
d_p=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 12.5 for the cefpiramide peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for cefpiramide and the peak for cefpiramide lactone (generated in situ) is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_r in percent of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′).* Calculate the capacity (k') for cefpiramide as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:
t_r=Retention time of cefpiramide in minutes; and
t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:
D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (k') for cefpiramide is satisfactory if it is not less than 2.0 and not more than 3.0. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of cefpiramide per milligram of sample as follows:

$$\text{Micrograms of cefpiramide per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:
A_u=Area of the cefpiramide peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the cefpiramide peak in the chromatogram of the cefpiramide working standard;
P_s=Cefpiramide activity in the cefpiramide working standard solution in micrograms per milliliter;
C_u=Milligrams of cefpiramide sample per milliliter of sample solution; and
m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 5 milligrams of cefpiramide per milliliter.

(4) *Total related substances.* Proceed as directed in paragraph (b)(1) of this section except use the following reagents, standard and sample solutions, and calculations:

(i) *Reagents*—(A) *0.03M phosphate buffer.* Dissolve 4.08 grams of monobasic potasssium phosphate in 800 milliliters of water. Adjust the pH to 7.5 with 1*N* sodium hydroxide and dilute to 1,000 milliliters with water.

(B) *Mobile phase.* Mix 0.03*M* phosphate buffer: methanol (750:250). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions.*

(A) *Working standard solution.* Transfer about 12.5 milligrams of 5-mercapto-1-methyl-1*H*-tetrazole (MMT) and an amount of cefpiramide working standard equivalent to about 25 milligrams of cefpiramide activity, both accurately weighed, to a 100-milliliter volumetric flask. Dissolve and dilute to volume with 0.03*M* phosphate buffer. Further dilute 2.0 milliliters of this solution to 100 milliliters with mobile phase.

(B) *Sample solution.* Transfer about 25 milligrams of the test material, accurately weighed, to a 50-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(iii) *Calculations.* Calculate the percentages, individually, of MMT and any other compounds detected as follows:

$$T_1 = \text{Percent MMT (tetrazole)} = \frac{A_u \times C_s \times P_s \times 100}{A_s \times C_u \times 1{,}000}$$

$$T_2 = \text{Percent related compound} = \frac{R_u \times C_s \times P_s \times 100}{R_s \times C_u \times 1{,}000}$$

$$\text{L} = \text{Percent largest related compound} = \frac{L_u \times C_s \times P_s \times 100}{R_s \times C_u \times 1{,}000}$$

where:

A_u=Area of the tetrazole sample peak;

A_s=Area of the tetrazole working standard peak;

C_s=Concentration of the working standard in milligrams per milliliter;

P_s=Potency of the working standard in micograms per milligram;

C_u=Concentration of the sample solutions in milligrams per milliliter;

R_u=Sum of peak areas of other compounds, excepting MMT and cefpiramide, detected in the sample chromatogram.

R_s=Area of the cefpiramide working standard peak; and

L_u=Area of the largest related peak, except MMT.

T=Percent total related compounds=$T_1 + T_2$.

(5) *Specific rotation*. Dilute an accurately weighed sample with sufficient dimethylformamide to obtain a concentration of approximately 10 milligrams of cefpiramide per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(6) *Identify*. Proceed as directed in § 436.211 of this chapter using a 1-percent potassium bromide disc prepared as directed in § 436.211(b)(1).

(7) *Chrystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[55 FR 14240, Apr. 17, 1990]

§ 442.69 Cefmetazole.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefmetazole is (6*R*,7*S*)-7-[2-[(cyanomethyl)thio]acetamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of cefmetazole activity per milligram.

(ii) Its moisture content is not more than 0.5 percent.

(iii) It gives a positive identity test for cefmetazole.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 442.70a(b)(1).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *Identity*. Proceed as directed in § 436.211 of this chapter using a mineral oil mull prepared as described in paragraph (b)(2) of that section.

[59 FR 12546, Mar. 17, 1994]

§ 442.70a Sterile cefmetazole sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile cefmetazole sodium is the sodium salt of (6*R*-cis)-7-[[[cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is a lyophilized powder. It is so purified and dried that:

(i) If the cefmetazole sodium is not packaged for dispensing, its

cefmetazole potency is not less than 860 micrograms and not more than 1,003 micrograms of cefmetazole activity per milligram on an anhydrous basis. If the cefmetazole sodium is packaged for dispensing, its cefmetazole potency is not less than 860 micrograms and not more than 1,003 micrograms of cefmetazole activity per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefmetazole that it is represented to contain.

(ii) It is sterile.

(iii) It contains not more than 0.2 endotoxin units per milligram.

(iv) Its moisture content is not more than 0.5 percent.

(v) The pH of an aqueous solution containing 100 milligrams per milliliter is not less than 4.2 and not more than 6.2.

(vi) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefmetazole potenty and content (if packaged for dispensing), sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(B) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 214 nanometers, a 25-centimeter X 4.0- or 4.6-millimeter (inside diameter) column packed with microparticulate (5 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded to silicas, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Transfer 5.75 grams of ammonium dihydrogen phosphate to a 1-liter container. Add 700 milliliters of deionized water and agitate to aid dissolution. Transfer 3.2 milliliters of 40 percent tetrabutylammonium hydroxide (TBAH) in distilled water to the solution and shake. Add 280 milliliters of methanol and a range 20 to 30 milliliters of tetrahydrofuran and mix well. Adjust the pH to 4.5±0.1 with phosphoric acid. The mobile phase is 0.05*M* ammonium dihydrogen phosphate: methanol: tetrahydrofuran (700:280:20–30). It is 0.005*M* with respect to TBAH. Filter the mobile phase through a suitable filter capable of removing particulate matter to 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve and dilute and accurately weighed portion of the cefmetazole working standard in sufficient mobile phase to obtain a solution containing 0.2 milligram of cefmetazole activity per milliliter. Analyze this solution within 10 minutes.

(B) *Sample solutions*—(*1*) *Product not packaged for dispensing (micrograms of cefmetazole per milligram).* Dissolve an accurately weighed sample with sufficient mobile phase to obtain a solution containing approximately 0.2 milligram of cefmetazole per milliliter (estimated). Analyze this solution within 10 minutes.

(*2*) *Product packaged for dispensing.* Determine both micrograms of cefmetazole per milligram of sample and milligrams of cefmetazole per container. Use separate containers for

preparation of each sample solution as described in paragraphs (b)(1)(ii)(B)(*2*)(*i*) and (*ii*) of this section.

(*i*) Micrograms *of cefmetazole per milligram.* Dissolve an accurately weighed sample with sufficient mobile phase to obtain a solution containing approximately 0.2 milligram of cefmetazole per milliliter (estimated). Analyze this solution within 10 minutes.

(*ii*) *Milligrams of cefmetazole per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 1,000 micrograms of cefmetazole activity per milliliter (estimated). Further dilute this solution with mobile phase to obtain a solution containing 0.2 milligram of cefmetazole activity per milliliter (estimated). Analyze this solution within 10 minutes.

(C) *Resolution test solution.* Dissolve an accurately weighed portion of cefmetazole working standard in 0.01*N* sodium hydroxide to obtain a solution containing approximately 1.0 milligram of cefmetazole activity per milliliter. Heat this solution at 95 °C for 10 minutes. This procedure generates cefmetazole lactone. Dilute 1.0 milliliter of this solution to 20 milliliters with mobile phase.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 10 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and
b=Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 0.94 and not more than 1.6.

(B) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in § 436.216(*c*)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;
n = Number of theoretical plates; and
d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 20 for the cefmetazole peak.

(C) *Resolution factor.* The resolution factor (*R*) between the peak for cefmetazole and the peak for cefmetazole lactone (generated in situ) is satisfactory if it is not less than 3.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′).* Calculate the capacity factor (*k′*) for cefmetazole as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of cefmetazole in minutes; and
t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (*k′*) for cefmetazole is satisfactory if it is not less than 2.0 and not more than 8.0. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*—(A) *Cefmetazole potency (micrograms of cefmetazole per milligram).* Calculate the micrograms of

cefmetazole per milligram of sample as follows:

$$\text{Micrograms of cefmetazole per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefmetazole peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefmetazole peak in the chromatogram of the cefmetazole working standard;

P_s=Cefmetazole activity in the cefmetazole working standard solution in micrograms per milliliter;

C_u=Milligrams of cefmetazole sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(B) *Cefmetazole content (milligrams of cefmetazole per container).* Calculate the cefmetazole content of the container as follows:

$$\text{Milligrams of cefmetazole per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefmetazole peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefmetazole peak in the chromatogram of the cefmetazole working standard;

P_s=Cefmetazole activity in the cefmetazole working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(3) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia bacterial endotoxins test.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter using a mineral oil mull prepared as described in § 436.211(b)(2).

[55 FR 6634, Feb. 26, 1990]

§ 442.80 Cefprozil.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefprozil is an approximate 9:1 mixture of the Z (cis) and the E (trans) isomers, respectively, of (6*R*,7*R*)-7-[(*R*)-2-amino-2-(*p*-hydroxyphenyl)acetamido]8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms nor more than 1,050 micrograms of cefprozil activity per milligram, on an anhydrous basis.

(ii) The ratio of its (E) isomer to total cefprozil is not less than 0.06 nor more than 0.11.

(iii) Its moisture content is not less than 3.5 percent nor more than 6.5 percent.

(iv) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 3.5 nor more than 6.5.

(v) It is crystalline.

(vi) It gives positive identity tests.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cefprozil potency, E isomer to total cefprozil ratio, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 25 centimeter × 3.9 to 4.6 millimeter (id) column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded to silicas, a flow rate of 1.0 milliliter per minute, and a known injection volume of 10 microliters. The retention time for cefprozil (Z) is between 4 and 6 minutes and the retention time for cefprozil (E) is between 6 and 8 minutes. Mobile phase, working standard

and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Dissolve 20.7 grams of ammonium phosphate, monobasic in 1,800 milliliters of water and adjust the pH to 4.4 with phosphoric acid, if necessary. Add 200 milliliters of acetonitrile and mix. Filter the mobile phase through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph. The proportion of acetonitrile may be modified in the range of 6 to 14 percent to obtain the desired retention times. Increasing the amount of acetonitrile will decrease both the retention times and the separation between the isomers, whereas, decreasing the amount of acetonitrile will increase retention times and the separation between the isomers.

(ii) *Preparation of working standard solutions*—(A) *Cefprozil (Z) working standard solution.* Accurately weigh approximately 12.5 milligrams of the cefprozil (Z) working standard into a 50-milliliter volumetric flask. Dilute to volume with water and shake the flask vigorously until the solute dissolves completely. Use this solution within 6 hours.

(B) *Cefprozil (E) working standard solution.* Accurately weigh approximately 12.5 milligrams of the cefprozil (E) working standard into a 50-milliliter volumetric flask. Dilute to volume with water and shake the flask vigorously until the solute dissolves completely. Pipet 5 milliliters into a 50-milliliter volumetric flask, dilute to volume with water and mix thoroughly. Use this solution within 6 hours.

(iii) *Sample solution.* Accurately weigh approximately 15 milligrams of sample into a 50-milliliter volumetric flask. Dilute to volume with water and shake the flask vigorously until the solute dissolves completely. Use this solution within 6 hours.

(iv) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_S)is satisfactory if it is not less than 0.9 and not more than 1.1 for the cefprozil (Z) response.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 10 for the cefprozil (Z) response.

(C) *Resolution factor.* The resolution factor (R) between the response for cefprozil (Z) and the response for cefprozil (E) is satisfactory if it is not less than 2.5.

(D) *Coefficient of variation (Relative standard deviation).* The coefficient of variation ($S_R$of 5 replicate injections of the cefprozil (Z) reference solution response) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k').* The capacity factor (k') for cefprozil (Z) is satisfactory if it is not less than 0.7 and not more than 1.1. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(v) *Calculations.* Calculate the micrograms of cefprozil per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of cefprozil (Z) or cefprozil (E) per milligram (as is)} = \frac{A_u \times P_s}{A_s \times C_u}$$

$$\text{Micrograms of cefprozil per milligram (as is)} = \frac{\text{cefprozil (Z)} + \text{cefprozil (E)}}{}$$

$$\text{Micrograms of cefprozil per milligram (Anhydrous)} = \frac{\text{cefprozil potency (as is)} \times 100}{(100 - m)}$$

where:

A_U = Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the cefprozil (Z) or the cefprozil (E) working standard ;

P_S = Cefprozil (Z) or cefprozil (E) activity in the cefprozil (Z) or the cefprozil (E) working standard solution in micrograms per milliliter;

C_U = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Cefprozil (E)/cefprozil ratio.* Using the procedure described in paragraph (b)(1) of this section calculate the cefprozil (E)/cefprozil ratio as follows:

$$\text{Trans ratio} = \frac{\text{cefprozil (E) (mcg/mg, as is)}}{\text{cefprozil (mcg/mg, as is) Total}}$$

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a carbon dioxide free aqueous solution containing 5 milligrams of cefprozil per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Identity*—(i) *Infrared.* Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(ii) *High performance liquid chromatography (HPLC).* The HPLC retention times for the responses of the cefprozil isomers in the assay preparation of the sample must be within 2 percent of the HPLC retention times of the responses of the corresponding cefprozil working standards.

[58 FR 26660, May 4, 1993]

Subpart B—Oral Dosage Forms

§ 442.104 Cefaclor monohydrate oral dosage forms.

§ 442.104a Cefaclor monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefaclor monohydrate capsules are composed of cefaclor monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cefaclor monohydrate equivalent to either 250 milligrams or 500 milligrams of cefaclor. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefaclor that it is represented to contain. Its moisture content is not more than 8.0 percent. The cefaclor monohydrate used conforms to the standards prescribed by § 442.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefaclor monohydrate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cefaclor monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(i) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cefaclor working standard in sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter.

(ii) *Preparation of sample solution.* Place one capsule into a high-speed glass blender jar containing sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter. Filter a portion to be used through a 10-micron filter.

(iii) *Calculations.* Calculate the cefaclor content in milligrams per capsule as follows:

$$\text{Milligrams of cefaclor per capsule} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u = Absorbance of sample solution;

P_a = Potency of working standard in micrograms per milliliter;

A_s = Absorbance of working standard solution;

d = Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[46 FR 3833, Jan. 16, 1981; 46 FR 21360, Apr. 10, 1981]

§ 442.104b Cefaclor monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefaclor monohydrate for oral suspension is cefaclor monohydrate with one or more suitable

and harmless diluents, buffer substances, colorings and flavorings. When reconstituted as directed in the labeling, each milliliter contains cefaclor monohydrate equivalent to 25 milligrams, 37.5 milligrams, 50 milligrams, or 75 milligrams of cefaclor. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefaclor that it is represented to contain. Its moisture content is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 5.0. The cefaclor monohydrate used conforms to the standards prescribed by § 442.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefaclor monohydrate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The cefaclor monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(i) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cefaclor working standard in sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter.

(ii) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling. Transfer a 5.0-milliliter portion into an appropriate-sized volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 4.5 (as described in § 436.101(a)(4) of this chapter) to obtain a concentration of 1 milligram of cefaclor per milliliter.

(iii) *Calculations.* Calculate the cefaclor content as follows:

$$\begin{array}{c}\text{Milligrams of cefaclor}\\ \text{for 5 milliliters of sample}\end{array} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Absorbance of sample solution;
P_a=Potency of working standard in micrograms per milliliter;
A_s=Absorbance of working standard solution;
d=Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[46 FR 3833, Jan. 16, 1981; 46 FR 21360, Apr. 10, 1981, as amended at 47 FR 22515, May 25, 1982; 54 FR 41824, Oct. 12, 1989]

§ 442.106 Cefadroxil monohydrate oral dosage forms.

§ 442.106a Cefadroxil monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil monohydrate capsules are composed of cefadroxil monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains either 250 or 500 milligrams of cefadroxil. Its moisture content is not more than 7.0 percent. The cefadroxil monohydrate used conforms to the standards prescribed by § 442.6(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefadroxil monohydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cefadroxil monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 20.0 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 442.40(b)(1)(ii) of this chapter, preparing the sample as follows: Blend a representative number of capsules in a high-speed glass blender jar with sufficient distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

[43 FR 20978, May 16, 1978; 43 FR 27180, June 23, 1978; 45 FR 16472, Mar. 14, 1980. Redesignated and amended at 46 FR 2992, Jan. 13, 1981; 50 FR 19919, May 13, 1985]

§ 442.106b Cefadroxil monohydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefadroxil monohydrate tablets are composed of cefadroxil monohydrate and one or more suitable and harmless binders and lubricants, and with or without coloring and film-coating substances. Each tablet contains cefadroxil monohydrate equivalent to 1,000 milligrams of cefadroxil. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefadroxil that it is represented to contain. Its moisture content is not more than 8.0 percent. The tablets disintegrate within 15 minutes. The cefadroxil monohydrate used conforms to the standards prescribed by § 442.6(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefadroxil monohydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The cefadroxil monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 20 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the cefadroxil content as follows:

(*a*) *Preparation of working standard solution*. Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to a final concentration of 1 milligram of cefadroxil per milliliter.

(*b*) *Preparation of sample solution*. Blend a representative number of tablets in a high-speed glass blender jar

with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(*c*) *Calculations.* Calculate the cefadroxil content as follows:

$$\text{Milligrams per tablet} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Absorbance of sample solution;
P_s=Potency of working standard in micrograms per milligram;
d=Dilution factor for sample;
A_s=Absorbance of working standard solution;
n=Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[46 FR 2992, Jan. 13, 1981; 46 FR 15880, Mar. 10, 1981, as amended at 50 FR 19919, May 13, 1985; 54 FR 47352, Nov. 14, 1989]

§442.106c Cefadroxil monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil monohydrate for oral suspension is cefadroxil monohydrate with one or more suitable and harmless preservatives, suspending agents, surfactants, binders, and flavorings. When reconstituted as directed in the labeling, each milliliter contains cefadroxil monohydrate equivalent to either 25, 50, or 100 milligrams of cefadroxil. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefadroxil that it is represented to contain. Its moisture content is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.0. The cefadroxil monohydrate used conforms to the standards prescribed by §442.6(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefadroxil monohydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The cefadroxil monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1). Further dilute an aliquot of this solution with solution 1 to the reference concentration of 20.0 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in §442.40(b)(1)(ii) of this chapter, except prepare the working standard and sample solutions and calculate the cefadroxil content as follows:

(*a*) *Preparation of working standard solution.* Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to a final concentration of 1 milligram of cefadroxil per milliliter.

(*b*) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion to a volumetric flask and bring to volume with distilled water to give a stock solution of convenient concentration.

Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(*c*) *Calculations.* Calculate the cefadroxil content as follows:

$$\text{Milligrams per dose} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:
A_u=Absorbance of sample solution;
P_s=Potency of working standard in micrograms per milligram;
d=Dilution factor for sample;
A_s=Absorbance of working standard solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[46 FR 16679, Mar. 13, 1981, as amended at 50 FR 19919, May 13, 1985]

§ 442.107 Cefadroxil hemihydrate oral dosage forms.

§ 442.107a Cefadroxil hemihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil hemihydrate capsules are composed of cefadroxil hemihydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cefadroxil hemihydrate equivalent to 500 milligrams of cefadroxil. Its cefadroxil content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefadroxil that it is represented to contain. Its moisture content is not more than 7.0 percent. It passes the dissolution test. The cefadroxil hemihydrate used conforms to the standards prescribed in § 442.7(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefadroxil hemihydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(B) The batch for content, moisture, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefadroxil hemihydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Cefadroxil content.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 20 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cefadroxil.* Proceed as directed in § 442.40(b)(1)(ii), except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(A) *Preparation of working standard solutions.* Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter.

(B) *Preparation of sample solutions.* Blend a representative number of capsules in a high-speed glass blender jar with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(C) *Calculations.* Calculate the cefadroxil content as follows:

$$\text{Milligrams of cefadroxil per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:
A_U = Absorbance of sample solution;
A_S = Absorbance of working standard solution;
P_S = Potency of working standard solution in micrograms per milliliter;
d = Dilution factor of the sample;
n = Number of capsules in the sample assayed.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in §436.215 of this chapter. The quantity Q (the amount of cefadroxil dissolved) is 75 percent within 45 minutes.

[59 FR 8857, Feb. 24, 1994]

§442.107b Cefadroxil hemihydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefadroxil hemihydrate tablets are composed of cefadroxil hemihydrate and one or more suitable and harmless binders and lubricants, with or without coloring and film-coating substances. Each tablet contains cefadroxil hemihydrate equivalent to 1,000 milligrams of cefadroxil. Its cefadroxil content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefadroxil that it is represented to contain. Its moisture content is not more than 8.0 percent. It passes the dissolution test. The cefadroxil hemihydrate used conforms to the standards prescribed in §442.7(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefadroxil hemihydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(B) The batch for content, moisture, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefadroxil hemihydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Cefadroxil content.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 20 micrograms of cefadroxil per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay for cefadroxil.* Proceed as directed in §442.40(b)(1)(ii), except prepare the working standard and sample solutions and calculate the potency of the sample as follows:

(A) *Preparation of working standard solutions.* Dissolve and dilute an accurately weighed portion of the cefadroxil working standard in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter.

(B) *Preparation of sample solutions.* Blend a representative number of tablets in a high-speed glass blender jar with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cefadroxil per milliliter (estimated).

(C) *Calculations.* Calculate the cefadroxil content as follows:

$$\text{Milligrams of cefadroxil per tablet} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:
A_U = Absorbance of sample solution;
A_S = Absorbance of working standard solution;

P_S = Potency of working standard solution in micrograms per milliliter;
d = Dilution factor of the sample; and
n = Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of cefadroxil dissolved) is 75 percent within 30 minutes.

[59 FR 8857, Feb. 24, 1994]

§ 442.115 Cefixime trihydrate oral dosage forms.

§ 442.115a Cefixime trihydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefixime trihydrate for oral suspension is cefixime trihydrate with one or more suitable and harmless preservatives, suspending agents, diluents, and flavorings. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 20 milligrams of cefixime. Its cefixime trihydrate potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefixime that it is represented to contain. Its moisture content is not more than 2.0 percent. When reconstituted as described in labeling, the pH of the suspension is not less than 2.5 and not more than 4.5. It passes the identity test for the presence of the cefixime moiety. The cefixime trihydrate used conforms to the standards prescribed by § 442.15(a)(1) of this part.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 436.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefixime trihydrate used in making the batch, for potency, moisture, pH, crystallinity, specific rotation, and identity.

(B) The batch, for content, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research.

(A) The cefixime used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Content.* Proceed as directed in § 442.15(b)(1) of this part, preparing the sample solution and calculating the cefixime content as follows:

(i) *Preparation of the sample solution.* Reconstitute as directed in the labeling. Transfer a 5.0-milliliter portion of the suspension into an appropriately sized volumetric flask and quantitatively dilute stepwise with 0.1*M* phosphate buffer, pH 7.0, to obtain a concentration of 0.2 milligram of cefixime activity per milliliter (estimated).

(ii) *Calculations.* Calculate the cefixime content as follows:

$$\text{Milligrams of cefixime per 5 milliliters of sample} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:
A_u = Area of the cefixime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s = Area of the cefixime peak in the chromatogram of the cefixime working standard.
P_s = Cefixime activity in the cefixime working standard solution in micrograms per milliliter; and
d = Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(4) *Identity.* The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the cefixime working standard.

[53 FR 24259, June 28, 1988]

§ 442.115b Cefixime trihydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefixime trihydrate tablets are composed of cefixime trihydrate and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet

contains cefixime trihydrate equivalent to either 200 milligrams or 400 milligrams of cefixime. Its cefixime trihydrate content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefixime that it is represented to contain. Its moisture content is not more than 10.0 percent. It passes the dissolution test. It passes the identity test for the presence of the cefixime moiety. The cefixime used conforms to the standards prescribed by § 442.15(a)(1) of this part.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefixime used in making the batch for potency, moisture, pH, crystallinity, specific rotation, and identity.

(B) The batch, for content, moisture, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research.

(A) The cefixime used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Content.* Proceed as directed in § 442.15(b)(1) of this part, preparing the sample solution and calculating the cefixime content as follows:

(i) *Preparation of sample solution.* Grind one or a known number of tablets using a mortar and pestle. Quantitatively transfer the ground tablet(s) into a suitable volumetric flask, sonicate and dilute with 0.1*M* phosphate buffer, pH 7.0 to a concentration of 4 milligrams per milliliter. Centrifuge the sample at 3,000 revolutions per minute for 10 minutes. Take an aliquot of the supernatant and qualitatively dilute to a concentration of 0.2 milligram of cefixime activity per milliliter in 0.1*M* phosphate buffer, pH 7.0 (estimated).

(ii) *Calculations.* Calculate the cefixime content as follows:

$$\text{Milligrams of cefixime per tablet} = \frac{A_u \times P_s \times d}{A_s \times n}$$

where:

A_u = Area of the cefixime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the cefixime peak in the chromatogram of the cefixime working standard.

P_s = Cefixime activity in the cefixime working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of tablets in the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of cefixime dissolved) is 75 percent within 45 minutes.

(4) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefixime working standard.

[53 FR 24259, June 28, 1988]

§ 442.119 Cefuroxime axetil oral dosage forms.

§ 442.119a Cefuroxime axetil tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime axetil tablets are composed of cefuroxime axetil and one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains 125 milligrams, 250 milligrams, or 500 milligrams of cefuroxime activity. Its potency is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefuroxime activity that it is represented to contain. Its moisture content is not more than 2.0 percent at the time of certification and not more than 6.0 percent at the time of expiry. It passes the dissolution test. It passes the film-coat rupture test. It passes the identity test. The cefuroxime axetil used conforms to the standards prescribed by § 442.19(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefuroxime axetil used in making the batch for potency, isomer A ratio, moisture, crystallinity, and identity.

(B) The batch for potency, moisture, dissolution, film-coat rupture, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefuroxime axetil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 442.19(b)(1). Working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve approximately 30 milligrams of the cefuroxime axetil working standard, accurately weighed, in methanol and dilute to 25 milliliters. Transfer 10.0 milliliters of the working standard solution to a 50-milliliter volumetric flask. Add 5.0 milliliters of internal standard solution, 3.8 milliliters of methanol, and dilute to volume with 0.2*M* ammonium phosphate solution to obtain a stock solution containing 0.24 milligram of cefuroxime axetil per milliliter. Store the stock solution under refrigeration no more than 8 hours.

(B) *Sample solution.* Grind a representative number of tablets in a mortar and pestle. Immediately swirl the ground tablets in a volumetric flask containing methanol and shake for 10 minutes to dissolve the ground cefuroxime axetil. Dilute with methanol to give a stock solution of convenient concentration. Filter the stock solution. Transfer 5.0 milliliters of filtrate to a 50-milliliter volumetric flask. Add 5.0 milliliters of internal standard solution and 8.8 milliliters of methanol. Dilute to volume with 0.2*M* ammonium phosphate solution. Store in a refrigerator and use within 8 hours.

(ii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory for isomer A if it is not more than 1.5 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory for isomer A if it is greater than 3,000 theoretical plates.

(C) *Resolution.* The resolution (*R*) between isomer A and isomer B of cefuroxime axetil is satisfactory if it is not less than 1.5 and the resolution (*R*) between isomer A and the delta-2 isomers of cefuroxime axetil is satisfactory if it is not less than 1.5.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(i)(B) of this section should not be changed.

(iii) *Calculations.* Calculate the cefuroxime content as follows:

$$\text{Milligrams of cefuroxime per tablet} = \frac{R_u \times P_s \times d}{R_s \times n}$$

where:

R_u=Sum of the peak heights of the cefuroxime axetil sample isomer A and isomer B peaks/Peak height of the internal standard;

R_s=Sum of the peak heights of the cefuroxime axetil working standard isomer A and isomer B peaks/Peak height of the internal standard;

P_s=Potency of the cefuroxime axetil working standard in milligrams of cefuroxime activity per milliliter;

d=Dilution factor of the sample; and

n=Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity *Q* (the amount of cefuroxime activity dissolved) is 60 percent at 15 minutes and 75 percent at 45 minutes.

(4) *Film-coat rupture test.* Proceed as directed in §436.217 of this chapter.

(5) *Identity.* The high-performance liquid chromatogram of the sample solution determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefuroxime axetil working standard solution.

[52 FR 42433, Nov. 5, 1987; 52 FR 45528, Nov. 30, 1987, as amended at 54 FR 47352, Nov. 14, 1989; 54 FR 50472, Dec. 6, 1989; 55 FR 11583, Mar. 29, 1990. Redesignated at 60 FR 27222, May 23, 1995]

§442.119b Cefuroxime axetil for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime axetil for oral suspension is cefuroxime axetil with one or more suitable and harmless diluents, suspending and sweetening agents, and flavorings. When reconstituted as directed in the labeling, it contains cefuroxime axetil equivalent to 25 milligrams of cefuroxime per millimeter. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cefuroxime that it is represented to contain. It passes the dissolution test. Its moisture content is not more than 0.2 percent. When reconstituted as directed in the labeling, its pH is not less than 3.5 and not more than 5.5. It passes the identity test. The cefuroxime axetil used conforms to the standards prescribed by §442.19(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefuroxime axetil used in making the batch for potency, isomer A ratio, moisture, crystallinity, and identity.

(B) The batch for cefuroxime potency, dissolution, moisture, pH of constituted suspension, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefuroxime axetil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 12 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §442.19(b)(1). Working standard and sample solutions and calculations are as follows:

(i) *Preparation of working standard solution.* Dissolve approximately 15 milligrams of the cefuroxime axetil working standard, accurately weighed, in 20.0 milliliters of methanol in a 50-milliliter volumetric flask. Dilute to volume with deionized water, and swirl to mix. Store for no more than 8 hours under refrigeration and protected from light.

(ii) *Preparation of sample solution.* Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the suspension equivalent to one dose into a 200-milliliter volumetric flask. Add 10 milliliters of methanol and disperse the sample. Dilute to volume with methanol. Dilute 20.0 milliliters of this solution to volume in a 50-milliliter volumetric flask with deionized water, swirl to mix, and allow to stand for 10 minutes. (Note: A white turbidity is formed.) Filter this solution via a suitable disposable filter unit, discarding the first 5 milliliters. Store for no more than 8 hours under refrigeration and protect from light.

(iii) *Calculations.* Calculate the milligrams of cefuroxime per dose (5 milliliters) as follows:

$$\text{Milligrams of cefuroxime per 5 milliliters of sample} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000}$$

where:

A_U = Sum of the areas of the cefuroxime axetil sample isomer A and isomer B peaks;

A_S = Sum of the peak areas of the cefuroxime axetil working standard isomer A and isomer B peaks;

P_S = Cefuroxime activity in the cefuroxime axetil working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Dissolution.* Proceed as directed in §436.215 of this chapter. The quantity Q (the amount of cefuroxime activity dissolved) is 60 percent at 30 minutes.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

(5) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefuroxime axetil working standard.

[60 FR 27222, May 23, 1995]

§ 442.121 Cephaloglycin dihydrate oral dosage forms.

§ 442.121a Cephaloglycin dihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate capsules are composed of cephaloglycin dihydrate and one or more suitable lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cephaloglycin dihydrate equivalent to 250 milligrams of cephaloglycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephaloglycin that it is represented to contain. Its moisture content is not more than 9 percent. The cephaloglycin used conforms to the standards prescribed by § 442.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephaloglycin dihydrate used in making the batch for potency, moisture, pH, cephaloglycin content, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cephaloglycin dihydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 4 to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19040, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 442.121b Cephaloglycin dihydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephaloglycin dihydrate for oral suspension is cephaloglycin dihydrate with one or more suitable diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains cephaloglycin dihydrate equivalent to 50 milligrams of cephaloglycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephaloglycin that it is represented to contain. Its moisture content is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 5.0. It passes the identity test for the presence of the cephaloglycin moiety. The cephaloglycin dihydrate used conforms to the standards prescribed by § 442.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephaloglycin dihydrate used in making the batch for potency, moisture, pH, cephaloglycin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The cephaloglycin dihydrate used in making the batch: 10 packages, each

containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Place an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4). Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10 micrograms of cephaloglycin per milliliter (estimated).

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(4) *Identity*. Dilute a representative portion of the sample with sufficient distilled water to give a concentration of 2.5 milligrams of cephaloglycin per milliliter (estimated). Shake vigorously on a mechanical shaker for 30 minutes. Filter through Whatman No. 1 filter paper, discarding the first few milliliters of filtrate. Further dilute an aliquot of the filtrate with sufficient distilled water to give a concentration of 0.05 milligram of cephaloglycin per milliliter (estimated). Using a suitable spectrophotometer, record the ultraviolet absorption spectrum of this solution from 230 to 320 nanometers. The spectrum compares qualitatively to that of the cephaloglycin working standard similarly treated.

[39 FR 19040, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§442.127 Cephalexin monohydrate oral dosage forms.

§442.127a Cephalexin monohydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cephalexin monohydrate tablets are composed of cephalexin monohydrate and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains cephalexin monohydrate equivalent to 250, 500, or 1,000 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not more than 9 percent. The tablets disintegrate within 30 minutes. The cephalexin monohydrate used conforms to the standards prescribed by §442.27(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephalexin monohydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The cephalexin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay*. Proceed as directed in §436.204 of this chapter, preparing the sample as follows: Blend a representative number of tablets in a high-speed glass blender with sufficient distilled water to give a stock solution of convenient concentration. Further

dilute with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10.0 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19040, May 30, 1974, as amended at 40 FR 49083, Oct. 21, 1975; 50 FR 19919, May 13, 1985; 52 FR 20710, June 3, 1987]

§ 442.127b Cephalexin monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate capsules are composed of cephalexin monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains cephalexin monohydrate equivalent to either 125, 250, or 500 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not more than 10 percent. The cephalexin monohydrate used conforms to the standards prescribed by § 442.27(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephalexin monohydrate used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The cephalexin monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Blend a representative number of capsules in a high-speed glass blender with sufficient distilled water to give a stock solution of convenient concentration. Further dilute with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10.0 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19040, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 442.127c Cephalexin monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin monohydrate for oral suspension is cephalexin monohydrate with one or more suitable and harmless diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains cephalexin monohydrate equivalent to 25 milligrams, 50 milligrams, or 100 milligrams of cephalexin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not

more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 6.0. The cephalexin used conforms to the standards prescribed by § 442.27(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephalexin used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The cephalexin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 1-percent potassium phosphate buffer, pH 6.0 (solution 1). Further dilute an aliquot of this solution with solution 1 to the reference concentration of 20.0 micrograms of cephalexin per milliliter (estimated).

(ii) *Iodometric assay.* Proceed as directed in § 436.204 of this chapter, preparing the sample as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion to a volumetric flask and bring to volume with distilled water. Further dilute an aliquot of this solution with distilled water to the prescribed concentration of cephalexin.

NOTE: The 10 milliliters of 0.01*N* iodine must be added within 20 seconds after the addition of the 2.0 milliliters of 1.2*N* hydrochloric acid, and the assay should be completed within 1 hour after the sample and standard are first put into solution.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 19040, May 30, 1974, as amended at 45 FR 16472, Mar. 14, 1980; 50 FR 19919, May 13, 1985]

§ 442.128 Cephalexin hydrochloride monohydrate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalexin hydrochloride monohydrate tablets are composed of cephalexin hydrochloride monohydrate and one or more suitable and harmless lubricants, colorings and coating substances. Each tablet contains cephalexin hydrochloride monohydrate equivalent to 250 milligrams, 333 milligrams or 500 milligrams of cephalexin. Its cephalexin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephalexin that it is represented to contain. Its moisture content is not more than 8.0 percent. The tablets pass the dissolution test. It passes the identity test. The cephalexin hydrochloride monohydrate used conforms to the standards prescribed by § 442.28(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cephalexin hydrochloride monohydrate used in making the batch for cephalexin potency, moisture, pH, identity, and crystallinity.

(B) The batch for cephalexin content, moisture, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research.

(A) The cephalexin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Cephalexin content.* Proceed as directed in § 442.140c(b)(1)(ii), except that ''cephalexin'' is substituted at each occurrence of ''cephradine''.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity *Q* (the amount of cephalexin dissolved) is not less than 75 percent at 45 minutes.

(4) *Identity.* Proceed as directed in § 436.367 of this chapter.

[54 FR 48860, Nov. 28, 1989]

§ 442.140a Cephradine for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine for oral suspension is cephradine with one or more suitable and harmless diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains 25 milligrams or 50 milligrams of cephradine. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of cephradine that it is represented to contain. Its moisture content is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.5 and not more than 6.0. The cephradine used conforms to the standards prescribed by § 442.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephradine used in making the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The cephradine used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the suspension into an appropriate-sized volumetric flask and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii) of this chapter, preparing the sample as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion to a volumetric flask and bring to volume with distilled water. Further dilute an aliquot of this solution with distilled water to 1 milligram of cephradine per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[40 FR 26272, June 23, 1975, as amended at 45 FR 16476, Mar. 14, 1980; 50 FR 19919, May 13, 1985]

§ 442.140b Cephradine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine capsules are composed of cephradine and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains 250 milligrams or 500 milligrams of cephradine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephradine that it is represented to contain. Its loss on drying is not more than 7.0 percent. The cephradine used conforms to the standards prescribed by § 442.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephradine used in making the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The cephradine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) the batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in §442.40(b)(1)(ii) of this chapter, preparing the sample as follows: Blend a representative number of capsules in a high-speed glass blender jar with sufficient distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to 1 milligrams of cephradine per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[40 FR 26272, June 23, 1975, as amended at 50 FR 19919, May 13, 1985]

§442.140c Cephradine tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine tablets are composed of cephradine and one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains 1,000 milligrams of cephradine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephradine that it is represented to contain. Its moisture content is not more than 6.0 percent. It disintegrates within 30 minutes. The cephradine used conforms to the standards prescribed by §442.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephradine used in making the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The cephradine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in §442.40(b)(1)(ii), except prepare the sample and calculate the cephradine content as follows:

(*a*) *Preparation of sample.* Blend a representative number of tablets in a high-speed glass blender jar with sufficient distilled water to give a stock solution of convenient concentration.

Further dilute an aliquot of this solution with distilled water to 1 milligram of cephradine per milliliter (estimated).

(*b*) *Calculations.* Calculate the cephradine content as follows:

$$\text{Milligrams per tablet} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Absorbance of sample solution;
P_s=Potency of working standard in micrograms per milligram;
d=Dilution factor for sample;
A_s=Absorbance of working standard solution;
n=Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[45 FR 22919, Apr. 4, 1980, as amended at 50 FR 19919, May 13, 1985]

§ 442.141 Cephradine dihydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephradine dihydrate capsules are composed of cephradine dihydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains 250 milligrams or 500 milligrams of cephradine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cephradine that it is represented to contain. Its moisture content is not more than 11.0 percent. It passes the dissolution test if the quantity *Q* is 85 percent at 60 minutes. The cephradine dihydrate used conforms to the standards prescribed by § 442.41(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephradine dihydrate used in making the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and dissolution.

(ii) Samples required:

(*a*) The cephradine dihydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute and aliquot of the stock solution with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 442.40(b)(1)(ii), except prepare the sample solution and calculate the cephradine content as follows:

(*a*) *Preparation of sample solution.* Blend a representative number of capsules in a high-speed glass blender jar with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of this solution with distilled water to a concentration of 1 milligram of cephradine per milliliter (estimated).

(*b*) *Calculations.* Calculate the cephradine content as follows:

$$\text{Milligrams per capsule} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Absorbance of sample solution;
P_s=Potency of working standard in micrograms per milligram;
d=Dilution factor for sample;
A_s=Absorbance of working standard solution;
n=Number of capsules in the sample assayed.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in §436.541 of this chapter, except:

(i) A distance of 2.5±0.2 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during test rather than 4.5±0.5 centimeters as specified in paragraph (a) of that section; and

(ii) In lieu of paragraph (d) of that section, use the interpretation described in the United States Pharmacopeia XX dissolution test.

[47 FR 11857, Mar. 19, 1982, as amended at 50 FR 19919, May 13, 1985]

§442.154 Cefpodoxime proxetil oral dosage forms.

§442.154a Cefpodoxime proxetil tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefpodoxime proxetil tablets are composed of cefpodoxime proxetil and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains cefpodoxime proxetil equivalent to either 100 milligrams or 200 milligrams of cefpodoxime. Its cefpodoxime proxetil content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefpodoxime that it is represented to contain. Its loss on drying is not more than 5 percent. It passes the dissolution test. It passes the identity test. The cefpodoxime proxetil used conforms to the standards prescribed by §442.54(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefpodoxime proxetil used in making the batch for potency, isomer ratio, moisture, and identity.

(B) The batch for content, loss on drying, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefpodoxime proxetil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Cefpodoxime content.* Proceed as directed in §442.54(b)(1), preparing the sample solution and calculating the cefpodoxime content as follows:

(i) *Preparation of sample solution.* Obtain the average tablet weight of at least 20 tablets. Grind the tablets using a mortar and pestle. Weigh approximately 660 milligrams into a suitable container. Add 30 milliliters of internal standard solution. Shake for 30 minutes using a horizontal platform shaker or equivalent. Centrifuge for about 10 minutes at 3,000 revolutions per minute until the particulate matter has settled. Withdraw a 1 milliliter aliquot of the supernatant and dilute with 9 milliliters of dilution solvent. The sample solutions are stable for at least 48 hours. Refrigeration is not recommended.

(ii) *Calculations.* Calculate the cefpodoxime content as follows:

$$\text{Milligrams of cefpodoxime per tablet} = \left(R_{sam}/R_{std}\right)\times\left(W_{std}/W_{sam}\right)\times\left(F_1/F_3\right)\times F_2\times F_4\times P$$

where:

R_{sam} = Ratio of cefpodoxime proxetil peaks area (sum of both epimers) to the internal standard peak area in the sample preparation;

R_{std} = Ratio of cefpodoxime proxetil peaks area (sum of both epimers) to the internal standard peak area in the standard preparation;

W_{std} = Weight of cefpodoxime proxetil reference standard, in milligrams;

W_{sam} = Weight of sample, in milligrams;

F_1 = Volume of internal standard used in the sample preparation, in milliliters;
F_2 = 0.766; The ratio of molecular weight for free-acid cefpodoxime over the molecular weight of cefpodoxime proxetil (427.46/557.61);
F_3 = Volume of internal standard used in the standard preparation, in milliliters;
F_4 = Average tablet weight, i.e., weight of tablets used in sample preparation divided by the number of tablets; and
P = Purity of the cefpodoxime proxetil reference standard, expressed as a decimal.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, except dry the sample at a temperature of 80° C and a pressure of 5 millimeters of mercury or less for 16 hours.

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of cefpodoxime activity dissolved) is 70 percent within 30 minutes.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention times for the peaks of the active ingredients must be within 2 percent of the retention times for the peaks of the corresponding reference standards.

[60 FR 58232, Nov. 27, 1995]

§ 442.154b Cefpodoxime proxetil granules for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefpodoxime proxetil granules for oral suspension is cefpodoxime proxetil and one or more suitable and harmless preservatives, sweeteners, suspending agents, buffers, and flavorings. When constituted as directed in the labeling, each milliliter contains the equivalent of either 10 or 20 milligrams cefpodoxime activity. Its cefpodoxime proxetil content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefpodoxime that it is represented to contain. Its loss on drying is not more than 0.5 percent. When constituted as described in the labeling, the pH of the suspension is not less than 4 and not more than 5.5. It passes the identity test. The cefpodoxime proxetil used conforms to the standards prescribed by § 442.54(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefpodoxime proxetil used in making the batch for potency, isomer ratio, moisture, and identity.

(B) The batch for content, loss on drying, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefpodoxime proxetil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 10 intermediate containers.

(b) *Tests and methods of assay*—(1) *Cefpodoxime content.* Proceed as directed in § 442.54(b)(1), preparing the sample solution and calculating the cefpodoxime content as follows:

(i) *Preparation of sample solution.* Reconstitute as directed in the labeling. Immediately before sampling the suspension, shake vigorously for several seconds. Into a suitable container, accurately weigh out 6 grams of the 50 milligrams per 5 milliliters suspension, or 3 grams of the 100 milligrams per 5 milliliters suspension. Add 5 milliliters of internal standard solution and 25 milliliters of dilution solvent. Shake for 30 minutes using a horizontal platform shaker or equivalent. Centrifuge for about 10 minutes at 3,000 revolutions per minute until the particulate matter has settled. Withdraw a 1 milliliter aliquot of the supernatant and dilute with 1 milliliter of dilution solvent. The sample solutions are stable for at least 48 hours. Refrigeration is not recommended.

(ii) *Calculations.* Calculate the cefpodoxime content as follows:

$$\text{Milligrams of cefpodoxime per 5 milliliters of suspension} = \left(R_{sam}/R_{std}\right)\times\left(W_{std}/W_{sam}\right)\times\left(F_1/F_3\right)\times\left(F_2/F_4\right)\times F_5\times P$$

where:

R_{sam} = Ratio of cefpodoxime proxetil peaks area (sum of both epimers) to the internal standard peak area in the sample preparation;

R_{std} = Ratio of cefpodoxime proxetil peaks area (sum of both epimers) to the internal standard peak area in the standard preparation;

W_{std} = Weight of cefpodoxime proxetil reference standard, in milligrams;

W_{sam} = Weight of sample, in grams;

F_1 = Volume of internal standard used in the sample; preparation, in milliliters;

F_2 = 0.766; The ratio of molecular weight for free-acid cefpodoxime over the molecular weight of cefpodoxime proxetil (427.46/557.61);

F_3 = Volume of internal standard used in the standard preparation, in milliliters;

F_4 = 0.2; Factor to convert to 5 milliliters;

F_5 = Specific gravity of suspension for milligram per 5 milliliter calculated on the air-free basis (specific gravity is determined on a sample of suspension that has been shaken gently on a platform shaker under vacuum for 2 hours); and

P = Purity of the cefpodoxime proxetil reference standard, expressed as a decimal.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, except dry the sample at a temperature of 80° C and a pressure of 5 millimeters of mercury or less for 16 hours.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug constituted as directed in the labeling.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention times for the peaks of the active ingredients must be within 2 percent of the retention times for the peaks of the corresponding reference standards.

[60 FR 58233, Nov. 27, 1995]

§ 442.180 Cefprozil oral dosage forms.

§ 442.180a Cefprozil tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefprozil tablets are composed of cefprozil and one or more suitable and harmless diluents, binders, lubricants, colorings, and coating substances. Each tablet contains cefprozil equivalent to either 250 milligrams or 500 milligrams of anhydrous cefprozil. The cefprozil content of the tablets is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of anhydrous cefprozil that it is represented to contain. The moisture content of the tablets is not more than 7 percent. The tablets pass the dissolution test. The tablets pass the identity tests. The cefprozil used conforms to the standards prescribed by § 442.80(a)(1) of this part.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefprozil used in making the batch for potency, E-isomer ratio, moisture, pH, crystallinity, and identity.

(B) The batch for content, moisture, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefprozil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Cefprozil content.* Proceed as directed in § 442.80(b)(1) of this part, preparing the sample solution and calculating the cefprozil content as follows:

(i) *Preparation of sample solution.* Place one or a known number of intact tablets into a 250-milliliter volumetric flask containing about 180 milliliters of distilled water. Allow the tablet(s) to disintegrate as aided by swirling and brief ultrasonication. Dilute the contents to volume with distilled water and mix thoroughly. Transfer an aliquot of this solution to a volumetric

flask of suitable size to obtain a solution containing 0.3 milligram per milliliter of cefprozil (estimated) when diluted to volume with water. Filter through a 0.45 micron filter prior to injection into the chromatographic system.

(ii) *Calculations.* Calculate the cefprozil content as follows:

$$\text{Milligrams of cefprozil (Z) or cefprozil (E) per tablet} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

$$\text{Milligrams of cefprozil per tablet} = \text{Milligrams of cefprozil (Z) per tablet} + \text{Milligrams of cefprozil (E) per tablet}$$

where:

A_U=Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the cefprozil (Z) or the cefprozil (E) working standard;

P_S=Cefprozil (Z) or cefprozil (E) activity in the cefprozil (Z) or the cefprozil (E) working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of tablets taken in the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of cefprozil activity dissolved) is 75 percent at 45 minutes.

(4) *Identity*—(i) *High performance liquid chromatography.* Using the high performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention times for the responses of the active ingredients must be within 2 percent of the retention times for the responses of the corresponding reference standards.

(ii) *Thin layer chromatography.* Proceed as directed in § 436.368 of this chapter.

[58 FR 26661, May 4, 1993]

§ 442.180b Cefprozil for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefprozil for oral suspension is cefprozil with one or more suitable and harmless preservatives, sweeteners, suspending agents, buffers, and flavorings. The cefprozil content of the oral suspension is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of anhydrous cefprozil that it is represented to contain. When constituted as directed in the labeling, each milliliter contains the equivalent of either 25 or 50 milligrams anhydrous cefprozil activity. Its moisture content is not more than 3 percent. When constituted as described in the labeling, the pH of the suspension is not less than 4.0 nor more than 6.0. It passes the identity tests. The cefprozil used conforms to the standards prescribed by § 442.80(a)(1) of this part.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefprozil used in making the batch for potency, E-isomer ratio, moisture, pH, crystallinity, and identity.

(B) The batch for content, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefprozil used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 10 intermediate containers.

(b) *Tests and methods of assay*—(1) *Cefprozil content.* Proceed as directed in § 442.80(b)(1), preparing the sample solution and calculating the cefprozil content as follows:

(i) *Preparation of sample solution.* Constitute as directed in the labeling. Transfer a portion of the suspension containing 250 milligrams (estimated) of cefprozil into a 250-milliliter volumetric flask using a glass syringe and a 13-gauge needle. Dilute to volume with water, ultrasonicate briefly to dissolve and mix well. Transfer a 15-milliliter aliquot of this solution to a 50-milliliter volumetric flask and dilute to volume with water to obtain a solution containing 0.3 milligram per

milliliter of cefprozil (estimated). Filter through a 0.45 micron filter prior to injection into the chromatographic system.

(ii) *Calculations.* Calculate the cefprozil content as follows:

$$\text{Milligrams of cefprozil (Z) or cefprozil (E) per 5 milliliters of sample} = \frac{A_u \times P_s \times d \times 5}{A_s \times 1{,}000 \times V}$$

$$\text{Milligrams of cefprozil per 5 mL of sample} = \begin{array}{c}\text{Milligrams of cefprozil (Z)/5 mL sample} \\ + \\ \text{Milligrams of cefprozil (E)/5 mL sample}\end{array}$$

where:

A_U=Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the cefprozil (Z) or cefprozil (E) response in the chromatogram of the cefprozil (Z) or the cefprozil (E) working standard;

P_S=Cefprozil (Z) or cefprozil (E) activity in the cefprozil (Z) or the cefprozil (E) working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

V = Volume of sample taken in milliliters.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug constituted as directed in the labeling.

(4) *Identity*—(i) *High Performance liquid chromatography.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention times for the responses of the active ingredients must be within 2 percent of the retention times for the responses of the corresponding reference standards.

(ii) *Thin layer chromatography.* Proceed as directed in § 436.368 of this chapter.

[58 FR 26661, May 4, 1993]

Subpart C—Injectable Dosage Forms

§ 442.208 Cefamandole nafate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefamandole nafate for injection is a dry mixture of cefamandole nafate and one or more suitable and harmless buffering agents. The cefamandole nafate may be isolated in the manufacture of cefamandole nafate for injection. Its cefamandole content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cefamandole that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 6.0 and not more than 8.0. If isolated, the cefamandole nafate used conforms to the standards prescribed by § 442.8a(a)(1). If the cefamandole nafate is not isolated, the potency of the dry mixture is not less than 810 micrograms and not more than 1,000 micrograms of cefamandole per milligram on an anhydrous basis when corrected for sodium carbonate; and the dry mixture gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) If isolated, the cefamandole nafate used in making the batch for cefamandole content, moisture, pH, and identity.

(*b*) The batch for cefamandole content, sterility, pyrogens, moisture, and pH. In addition, if the cefamandole nafate is not isolated, results of tests and assays on the dry mixture for potency and identity.

(ii) Samples required:

(*a*) For all tests except sterility: A minimum of 10 immediate containers, unless the cefamandole nafate is not isolated, a minimum of 15 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefamandole content.* Proceed as directed in § 436.324 of this chapter, preparing the sample solution and calculating the cefamandole content as follows:

(i) *Sample preparation.* Reconstitute the sample as directed in the labeling.

If it is represented as a single dose container, remove all of the withdrawable contents with a suitable hypodermic needle and syringe. If the labeling specifies the amount of cefamandole content in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with distilled water to obtain a concentration of 2.0 milligrams of cefamandole per milliliter (estimated). Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, add 30 milliliters of pH 2.3 buffer, dilute to volume with distilled water, and mix. In addition, if the cefamandole nafate is not isolated, prepare the sample solution as described in § 436.324(d) of this chapter. Determine the sodium carbonate content as follows: Dissolve an accurately weighed portion of the dry mixture, approximately 1.0 gram, with approximately 100 milliliters of distilled water. Titrate with 0.2*N* hydrochloric acid. Determine the end-point potentiometrically to the first equivalent using a glass calomel combination electrode. Each milliliter of 0.2*N* hydrochloric acid is equivalent to 21.2 milligrams of sodium carbonate.

(ii) *Calculations*—(*a*) Calculate the cefamandole content as follows:

$$\text{Milligrams of cefamandole} = \frac{A \times \begin{matrix}\text{Milligrams of}\\ \text{working standard.}\end{matrix} \times \begin{matrix}\text{Potency of working}\\ \text{standard in micrograms}\\ \text{per milligram}\end{matrix} \times f}{B \times 50 \times 1{,}000}$$

where:

A=The peak height of the sample;

B=The peak height of the working standard; and

f=The dilution factor of the sample.

(*b*) If the cefamandole nafate is not isolated in the manufacture of cefamandole nafate for injection, calculate the micrograms of cefamandole per milligram of sample as described in § 436.324(f) of this chapter. The micrograms per milligram of cefamandole is corrected for sodium carbonate content and moisture content.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefamandole per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter, except determine the pH 30 minutes after preparation of the sample solution.

(7) *Identity.* Proceed as directed in § 436.323 of this chapter.

[44 FR 20665, Apr. 6, 1979, as amended at 47 FR 42099, Sept. 24, 1982; 50 FR 19919, May 13, 1985]

§ 442.209 Cefamandole sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefamandole sodium for injection is a dry mixture of cefamandole sodium and one or more suitable and harmless buffering agents. Its cefamandole content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cefamandole that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 6.0 and not more than 8.5. The cefamandole sodium used conforms to the standards prescribed by § 442.9a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefamandole sodium used in making the batch for cefamandole content, moisture, pH, and identity.

(*b*) The batch for cefamandole content, sterility, pyrogens, moisture, and pH.

(ii) Samples required:

(*a*) The cefamandole sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefamandole content.* Proceed as directed in § 436.324 of this chapter, preparing the sample solution and calculating the cefamandole content as follows:

(i) *Sample solution.* Reconstitute the sample as directed in the labeling. If it is represented as a single-dose container, remove all the withdrawable contents with a suitable hypodermic needle and syringe. If the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with distilled water to obtain a concentration of 2.0 milligrams of cefamandole per milliliter (estimated). Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, add 30 milliliters of pH 2.3 buffer, dilute to volume with distilled water, and mix.

(ii) *Calculations.* Calculate the cefamandole content as follows:

$$\text{Milligrams of cefamandole} = \frac{A \times \begin{array}{c}\text{Milligrams of}\\ \text{working standard.}\end{array} \times \begin{array}{c}\text{Potency of working}\\ \text{standard in micrograms}\\ \text{per milligram}\end{array} \times f}{B \times 50 \times 1{,}000}$$

where:

A=The peak height of the sample;

B=The peak height of the working standard;

f=The dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cefamandole per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

[47 FR 20756, May 14, 1982, as amended at 50 FR 19919, May 13, 1985]

§ 442.211 Cefazolin sodium injectable dosage forms.

§ 442.211a Sterile cefazolin sodium.

The requirements for certification and the tests and methods of assay for sterile cefazolin sodium packaged for dispensing are described in § 442.11a, except for the following additional requirements if it is packaged with lidocaine hydrochloride injection 0.5 percent U.S.P.:

(a) The pH, when reconstituted and diluted to 100 milligrams per milliliter with lidocaine hydrochloride injection 0.5 percent U.S.P., is not less than 5.5 and not more than 7.0.

(b) In addition to the information required by § 442.11a (a)(3)(i), the following shall be submitted:

(1) The pH on the batch reconstituted with lidocaine hydrochloride injection 0.5 percent U.S.P.; and

(2) Results of tests and assays on the lidocaine hydrochloride injection 0.5 percent to show conformance with U.S.P. requirements.

[42 FR 18059, Apr. 5, 1977. Redesignated at 48 FR 33479, July 22, 1983]

§ 442.211b Cefazolin sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefazolin sodium injection is a frozen aqueous solution of cefazolin sodium in an isoosmotic diluent. Each milliliter contains cefazolin sodium equivalent to either 10 milligrams or 20 milligrams of cefazolin per milliliter. Its cefazolin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cefazolin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 7.0. It passes the identity test. The cefazolin used conforms to the standards prescribed by § 442.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Requests of tests and assays on:

(a) The cefazolin used in making the batch for cefazolin content, moisture, heavy metals, and identity.

(b) The batch for cefazolin content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(a) The cefazolin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefazolin content.* Proceed as directed in § 436.342 of this chapter, preparing the sample solution and calculating the cefazolin content as follows:

(i) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, transfer an accurately measured representative portion from each container, equivalent to 40 milligrams of cefazolin, to a 100-milliliter volumetric flask. Dilute to volume with buffer solution, pH 7.0, and mix. Transfer 10.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with buffer solution, pH 7.0, and mix

(ii) *Calculation.* Calculate the milligrams of cefazolin per milliliter of sample as follows:

$$\text{Milligrams of cefazolin per milliliter} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefazolin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard) /Area of internal standard peak;

R_s=Area of cefazolin peak in the chromatogram of the cefazolin working standard /Area of internal standard peak;

P_s=Cefazolin activity in the cefazolin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of cefazolin per kilogram.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefazolin working standard.

[48 FR 33479, July 22, 1983; 48 FR 40516, Sept. 8, 1983; 49 FR 48184, Dec. 11, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§442.212 Cefoperazone injectable dosage forms.

§442.212a Sterile cefoperazone sodium.

The requirements for certification and the tests and methods of assay for sterile cefoperazone sodium packaged for dispensing are described in §442.12a.

[48 FR 790, Jan. 7, 1983. Redesignated at 51 FR 36688, Oct. 15, 1986]

§442.212b Cefoperazone sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefoperazone sodium injection is a frozen aqueous iso-osmotic solution of cefoperazone sodium which may contain one or more suitable and harmless buffer substances in a diluent. Each milliliter contains cefoperazone sodium equivalent to either 20 milligrams or 40 milligrams of cefoperazone per milliliter. Its cefoperazone content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefoperazone that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The cefoperazone sodium used conforms to the standards prescribed by §442.12(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefoperazone sodium used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The cefoperazone sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*. Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Potency*. Proceed as directed in §436.338 of this chapter, preparing the sample solution and calculating the cefoperazone content as follows:

(i) *Sample solution*. Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with mobile phase to obtain a solution containing 160 micrograms per milliliter (estimated).

(ii) *Calculations*. Calculate the milligrams of cefoperazone per milliliter of sample as follows:

$$\text{Milligrams of cefoperazone per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefoperazone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefoperazone peak in the chromatogram of the cefoperazone working standard;

P_s=Cefoperazone activity in the cefoperazone working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in §436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 10 milligrams of cefoperazone per kilogram.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefoperazone working standard.

[51 FR 36688, Oct. 15, 1986, as amended at 54 FR 47352, Nov. 14, 1989; 55 FR 11583, Mar. 29, 1990]

§ 442.213 Cefotaxime injectable dosage forms.

§ 442.213a Sterile cefotaxime sodium.

The requirements for certification and the tests and methods of assay for sterile cefotaxime sodium packaged for dispensing are described in § 442.13a.

[46 FR 25607, May 8, 1981. Redesignated at 50 FR 45109, Oct. 30, 1985]

§ 442.213b Cefotaxime sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotaxime sodium injection is a frozen aqueous solution of cefotaxime sodium with one or more suitable and harmless buffer substances in an isosmotic diluent. Each milliliter contains cefotaxime sodium equivalent to either 20 milligrams or 40 milligrams of cefotaxime per milliliter. Its cefotaxime content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cefotaxime that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. It passes the identity test. The cefotaxime sodium used conforms to the standards prescribed by § 442.13(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefotaxime sodium used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The cefotaxime sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Potency.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 2.0 micrograms of cefotaxime per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, preparing the sample as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with distilled water to give a stock solution of convenient concentration. Further dilute with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of cefotaxime per kilogram.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identity.* Proceed as directed in § 436.323 of this chapter, except prepare spotting solutions as follows: Prepare solutions of the sample and working standard, each containing 1 milligram of cefotaxime per milliliter in distilled water.

[50 FR 45109, Oct. 30, 1985; 50 FR 48078, Nov. 21, 1985, as amended at 55 FR 11583, Mar. 29, 1990]

§442.214 Cefoxitin injectable dosage forms.

§442.214a Sterile cefoxitin sodium.

The requirements for certification and the tests and methods of assay for sterile cefoxitin packaged for dispensing are described in §442.14a.

[44 FR 10376, Feb. 20, 1979. Redesignated at 49 FR 47827, Dec. 7, 1984]

§442.214b Cefoxitin sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefoxitin sodium injection is a frozen aqueous solution of cefoxitin sodium with one or more suitable and harmless buffer substances in an isotonic diluent. Each milliliter contains cefoxitin sodium equivalent to either 20 or 40 milligrams of cefoxitin. Its cefoxitin content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefoxitin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 8.0. It passes the identity test. The cefoxitin sodium used conforms to the standards prescribed by §442.14(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cefoxitin sodium used in making the batch for cefoxitin content, moisture, pH, identity, and crystallinity.

(*b*) The batch for cefoxitin content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The cefoxitin sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Cefoxitin content.* Proceed as directed in §436.347 of this chapter, preparing the working standard and sample solutions and calculating the cefoxitin content as follows:

(i) *Working standard solution.* Dissolve an accurately weighed portion of the cefoxitin working standard with water to obtain a solution containing 200 micrograms of cefoxitin per milliliter.

(ii) *Sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient water to obtain a solution containing 200 micrograms of cefoxitin per milliliter (estimated).

(iii) *Calculations.* Calculate the milligrams of cefoxitin per milliliter of sample as follows:

$$\text{Milligrams of cefoxitin per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefoxitin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefoxitin peak in the chromatogram of the cefoxitin working standard;

P_s=Cefoxitin activity in the cefoxitin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of cefoxitin per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefoxitin working standard.

[49 FR 47827, Dec. 7, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.216 Ceftazidime injectable dosage forms.

§ 442.216a Ceftazidime pentahydrate for injection.

(a) *Requirements of certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftazidime pentahydrate for injection is a dry mixture of ceftazidime pentahydrate and sodium carbonate or *L*-arginine. Its ceftazidime potency is satisfactory if each milligram of ceftazidime pentahydrate for injection contains not less than 900 micrograms and not more than 1,050 micrograms of cefazidime activity when corrected for both loss on drying and its sodium carbonate or *L*-arginine content, as appropriate for the formulation. Its ceftazidime content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ceftazidime that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 12.5 percent if it contains *L*-arginine and not more than 13.5 percent if it contains sodium carbonate. The pH of its aqueous solution is not less than 5.0 and not more than 7.5. Its pyridine content, if it contains sodium carbonate, is not more than 0.4 percent, except that for the issuance of a certificate for each batch of the sodium carbonate formulation, the pyridine content is not more than 0.12 percent. Its pyridine content, if it contains *L*-arginine, is not more than 0.3 percent, except that for the issuance of a certificate, the pyridine content of the *L*-arginine formulation is not more than 0.10 percent. The ceftazidime pentahydrate conforms to the standard prescribed by § 442.16a(a)(1).

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, each package of the *L*-arginine formulation shall bear on its outside wrapper or container and on the immediate container the statement ''For Patients 12 years and Older''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ceftazidime pentahydrate used in making the batch for potency, loss on drying, pH, crystallinity, identity, and high molecular weight polymer content.

(*b*) The batch for ceftazidime potency, ceftazidime content, sterility, pyrogens, loss on drying, pH, and pyridine content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The ceftazidime pentahydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ceftazidime potency and content.* Determine both micrograms of ceftazidime per milligram of sample and milligrams of ceftazidime per container. Proceed as directed in § 442.16a(b)(1), preparing the sample solutions and calculating the potency and content as follows:

(i) *Preparation of sample solutions.* Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(i) (*a*) and (*b*) of this section.

(*a*) *Ceftazidime potency (micrograms of ceftazidime per milligram*). Accurately weigh and dissolve approximately 350 milligrams of ceftazidime sample in distilled water and dilute to volume in a 250-milliliter volumetric flask to obtain a stock solution containing approximately 1,000 micrograms of ceftazidime per milliliter. Mix well. Immediately prior to chromatography, further dilute 5 milliliters of stock solution to 50 milliliters with water to obtain a solution containing 100 micrograms of ceftazidime activity per milliliter (estimated).

(*b*) *Ceftazidime content (milligrams of ceftazidime per vial).* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of

potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with distilled water to obtain a concentration of 1.0 milligram per milliliter (estimated). Immediately prior to chromatography, dilute 5.0 milliliters of the sample solution to 50 milliliters with water.

(ii) *Calculations*—(*a*) *Ceftazidime potency (micrograms per milligram*). Calculate the micrograms of ceftazidime per milligram as follows:

$$\text{Micrograms of ceftazidime per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m - \text{S} - \text{A})}$$

where:

A_u = Area of the ceftazidime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the ceftazidime peak in the chromatogram of the ceftazidime working standard;

P_s = Ceftazidime activity in the ceftazidime working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution;

m = Percent loss on drying (determined as directed in § 436.200(h) of this chapter if the formulation contains sodium carbonate and determined as directed in § 436.200(g) of this chapter if the formulation contains *L*-arginine);

S = Percent sodium carbonate content of the sample (determined as directed in § 436.357 of this chapter); and

A = Percent *L*-arginine content of the sample (determined as directed in § 455.204 of this chapter, except use ceftazidime instead of aztreonam in the working standard solution and use water instead of mobile phase). Prepare the sample solution by diluting an accurately weighed portion of the contents of a vial with water to 0.2 milligram per milliliter (estimated). The resolution between the ceftazidime peak and the arginine peak is not less than 6.0, the asymmetry factor for the arginine peak is not more than 4.0).

(*b*) *Ceftazidime content (milligrams of ceftazidime per vial).* Calculate the ceftazidime content of the vial as follows:

$$\text{Milligrams of ceftazidime per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ceftazidime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftazidime peak in the chromatogram of the ceftazidime working standard;

P_s=Ceftazidime activity in the ceftazidime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 80 milligrams of ceftazidime per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(h) of this chapter if the formulation contains sodium carbonate and as directed in § 436.200(g) of this chapter if the formulation contains *L*-arginine.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, preparing the sample solution as follows: reconstitute the sample in the sealed container to give an aqueous solution containing approximately 100 milligrams per milliliter, relieving the pressure inside the container if necessary during the reconstitution.

(6) *Pyridine content.* Proceed as directed in § 436.358 of this chapter, using a temperature of 40 °C, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (5 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate of 1.6 milliliters per minute, and a known injection volume from 10 to 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *Phosphate buffer, pH 7.0.* Dissolve 5.68 grams of sodium phosphate, dibasic, anhydrous and 3.63 grams of potassium phosphate, monobasic, in water and dilute to 1,000 milliliters.

(*b*) *Mobile phase.* Mix 300 milliliters of acetonitrile and 100 milliliters of 0.25*M* ammonium phosphate, monobasic, dilute to 1,000 milliliters with water and add sufficient 10*M* ammonia solution to

give a pH of 7.0±0.1. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(*c*) *System suitability test solution.* Prepare a solution in phosphate buffer, pH 7.0, containing 25 micrograms of pyridine and 25 micrograms of an authentic sample of (6*R*, 7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(2-*t*-butoxycarbonylprop-2-yloxyimino) acetamido]-3-(1-pyridiniummethyl) ceph-3-em-4-carboxylate (*t*-butyl ceftazidime) per milliliter. Note, if no *t*-butyl ceftazidime is present in the sample solution, the working standard solution may be substituted for the system suitability test solution and the system suitability requirement for resolution for *t*-butyl ceftazidime is omitted.

(ii) *Preparation of working standard and sample solutions—(a) Working standard solution.* Accurately weigh approximately 250 milligrams of pyridine into a 100-milliliter volumetric flask and dilute to volume with water to obtain a stock solution containing approximately 2,500 micrograms of pyridine per milliliter. Mix well. Immediately prior to chromatography, further dilute 2.0 milliliters of stock solution to 200 milliliters with phosphate buffer, pH 7.0, to obtain a solution containing 25 micrograms of pyridine per milliliter.

(*b*) *Sample solution.* Accurately weigh approximately 660 milligrams of the sample into a 100-milliliter volumetric flask and add 50 milliliters of phosphate buffer, pH 7.0. Shake until dissolved and dilute to volume with phosphate buffer, pH 7.0. Mix well. Store the solution at a temperature below 15 °C and inject into the chromatograph within 1 hour of preparation.

(iii) *System suitability requirements—(a) Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2.5 at 5 percent of peak height.

(*b*) *Resolution.* The resolution (*R*) between the peak for pyridine and the peak for *t*-butyl ceftazidime is satisfactory if it is not less than 3.

(*c*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 3 percent.

If the system suitability requirements have been met, then proceed as described in § 436.358(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(6)(ii)(*b*) of this section should not be changed.

(iv) *Calculations.* Calculate the pyridine content in percent of the sample as follows:

$$\text{Pyridine content in percent} = \frac{H_u \times P_s \times 0.1}{H_s \times C_u}$$

where:

H_u=Height of the pyridine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

H_s=Height of the pyridine peak in the chromatogram of the pyridine working standard;

P_s=Pyridine content of the pyridine working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

[50 FR 48400, Nov. 25, 1985; 50 FR 52917, Dec. 27, 1985; 50 FR 53308, Dec. 31, 1985; 51 FR 2478, Jan. 17, 1986. Redesignated at 54 FR 40652, Oct. 3, 1989, and amended at 55 FR 11583, Mar. 29, 1990; 56 FR 484, Jan. 7, 1991; 59 FR 8398, Feb. 22, 1994]

§ 442.216b Ceftazidime sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftazidime sodium injection is a frozen, aqueous, iso-osmotic solution of ceftazidime sodium which may contain one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains ceftazidime sodium equivalent to 10, 20, or 40 milligrams of ceftazidime per milliliter. Its ceftazidime content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of ceftazidime that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5 It passes the

identity test. The ceftazidime pentahydrate conforms to the standards prescribed by §442.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The ceftazidime pentahydrate used in making the batch for potency, loss on drying, pH, crystallinity, identity, and high molecular weight polymer content.

(B) The batch for ceftazidime content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research;

(A) The ceftazidime pentahydrate used in making the batch: 10 packages, each containing 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Ceftazidime content.* Proceed as directed in §442.216(b)(1), except prepare the sample solution and calculate the ceftazidime content as follows:

(i) *Preparation of sample solution.* Remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature and dilute with mobile phase to obtain a solution containing 100 micrograms of ceftazidime per milliliter (estimated). Prepare the sample solution just prior to its introduction into the chromatograph.

(ii) *Calculation.* Calculate the milligrams of ceftazidime per milliliter of sample as follows:

$$\text{Milligrams of ceftazidime per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ceftazidime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftazidime peak in the chromatogram of the ceftazidime working standard;

P_s=Ceftazidime activity in the ceftazidime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, except inject a sufficient volume of the diluted solution to deliver 80 milligrams of ceftazidime per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identify.* The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ceftazidime working standard.

[54 FR 40652, Oct. 3, 1989]

§442.217 Ceftizoxime injectable dosage forms.

§442.217a Sterile ceftizoxime sodium.

The requirements for certification and the tests and methods of assay for sterile ceftizoxime sodium packaged for dispensing are described in §442.17a.

[48 FR 46272, Oct. 12, 1983. Redesignated at 49 FR 49286, Dec. 19, 1984]

§442.217b Ceftizoxime sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceftizoxime sodium injection is a frozen aqueous solution of ceftizoxime sodium with one or more suitable and harmless buffer substances in an isoosmotic diluent. Each milliliter contains ceftizoxime sodium equivalent to either 20 milligrams or 40 milligrams of ceftizoxime per milliliter. Ceftizoxime content is satisfactory if it is not less than 90 percent and not more than 115 percent of the represented number of milligrams of ceftizoxime. It is sterile. It is nonpyrogenic. Its pH is not less than

5.5 and not more than 8.0. It passes the identity test. The ceftizoxime sodium used conforms to the standards prescribed by § 442.17(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The ceftizoxime sodium used in making the batch for ceftizoxime content, moisture, pH, identity, and crystallinity.

(*b*) The batch for ceftizoxime content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research, of:

(*a*) The ceftizoxime sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assays.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Ceftizoxime content.* Proceed as directed in § 436.345 of this chapter, except prepare the sample solution and calculate the ceftizoxime content as follows:

(i) *Sample solution.* Using a suitable hypodermic needle and syringe, transfer an accurately measured representative portion from each container, equivalent to 40 milligrams of ceftizoxime, to a 100-milliliter volumetric flask. Dilute to volume with pH 7.0 buffer solution and mix. Transfer 10.0 milliliters of this solution to a 200-milliliter volumetric flask, add 5.0 milliliters of internal standard solution, dilute to volume with pH 7.0 buffer solution, and mix.

(ii) *Calculations.* Calculate the milligrams of ceftizoxime per milliliter of sample as follows:

$$\text{Milligrams of ceftizoxime per milliliter} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the ceftizoxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of the internal standard peak;

R_s=Area of the ceftizoxime peak in the chromatogram of the ceftizoxime working standard/Area of the internal standard peak;

P_s=Ceftizoxime activity in the ceftizoxime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of ceftizoxime per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ceftizoxime working standard.

[49 FR 49286, Dec. 19, 1984; 50 FR 253, Jan. 3, 1985, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.218 Cefuroxime injectable dosage forms.

§ 442.218a Sterile cefuroxime sodium.

The requirements for certification and the tests and methods of assay for sterile cefuroxime sodium packaged for dispensing are described in § 442.18a.

[48 FR 38461, Aug. 24, 1983. Redesignated at 54 FR 40654, Oct. 3, 1989]

§ 442.218b Cefuroxime sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefuroxime sodium injection is a frozen, aqueous, iso-osmotic solution of cefuroxime sodium which may contain one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter

contains cefuroxime sodium equivalent to 15 or 30 milligrams of cefuroxime per milliliter. Its cefuroxime content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefuroxime that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 5.0 and not more than 7.5. It passes the identity test. The cefuroxime sodium used conforms to the standards prescribed by § 442.18(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefuroxime sodium used in making the batch for potency, moisture, pH, and identity.

(B) The batch for cefuroxime content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefuroxime sodium used in making the batch: 10 packages, each containing 1 gram.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Cefuroxime content.* Proceed as directed in § 436.343 of this chapter, except prepare the sample solution and calculate the cefuroxime content as follows:

(i) *Preparation of sample solution.* Remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature and dilute with water to obtain a solution containing 50 micrograms of cefuroxime per milliliter (estimated). Prepare the sample solution just prior to its introduction in the chromatograph.

(ii) *Calculation.* Calculate the milligrams of cefuroxime per milliliter of sample as follows:

$$\text{Milligrams of cefuroxime per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of cefuroxime per kilogram.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefuroxime working standard.

[54 FR 40654, Oct. 3, 1989]

§ 442.220 Sterile cefonicid sodium.

The requirements for certification and the tests and methods of assay for sterile cefonicid sodium packaged for dispensing are described in § 442.20a.

[49 FR 34349, Aug. 30, 1984]

§ 442.222 Cefmenoxime hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefmenoxime hydrochloride for injection is a dry mixture of cefmenoxime hydrochloride and sodium carbonate. Each milligram of cefmenoxime hydrochloride for injection contains not less than 869 and not more than 1,015 micrograms of cefmenoxime on an anhydrous and sodium carbonate-free basis. Its

cefmenoxime content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cefmenoxime that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 1.5 percent. Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 6.4 and not more than 7.9. The cefmenoxime hydrochloride used conforms to the standards prescribed by § 442.22a(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefmenoxime hydrochloride used in making the batch for cefmenoxime content, moisture, identity, and crystallinity.

(B) The batch for cefmenoxime content, sterility, pyrogens, loss on drying, pH, and sodium carbonate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefmenoxime hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefmenoxime content.* Proceed as directed in § 436.363 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and a known injection volume between 10 and 20 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Phosphate buffer solution, pH 6.8.* Dissolve 6.4 grams of monobasic potassium phosphate and 18.9 grams of dibasic sodium phosphate in 750 milliliters of water. Adjust the pH to 6.8 with 1*N* sodium hydroxide and dilute to 1,000 milliliters.

(B) *Internal standard solution.* Dissolve and dilute 0.15 gram of phthalimide in methanol to 100 milliliters.

(C) *Mobile phase.* Mix water:acetonitrile:glacial acetic acid (50:10:1). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve approximately 50 milligrams of the cefmenoxime working standard, accurately weighed, in 10 milliliters of 0.1*M* phosphate buffer solution, pH 6.8 and dilute to 50 milliliters with mobile phase. Transfer 4.0 milliliters of this solution to a 50-milliliter volumetric flask, add 20 milliliters of internal standard solution and dilute to volume with mobile phase to obtain a solution containing 80 micrograms of cefmenoxime per milliliter.

(B) *Sample solutions.* Determine both micrograms of cefmenoxime per milligram of the sample and milligrams of cefmenoxime per container. Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(ii)(B) (*1*) and (*2*) of this section.

(*1*) *Micrograms of cefmenoxime per milligram.* Dissolve the accurately weighed dry contents of a sample with sufficient distilled water to obtain a solution containing 1 milligram of cefmenoxime per milliliter (estimated). Transfer 4.0 milliliters of this solution to a 50-milliliter volumetric flask, add 20 milliliters of internal standard solution and dilute to volume with mobile phase to obtain a solution containing 80 micrograms of cefmenoxime per milliliter (estimated).

(*2*) *Milligrams of cefmenoxime per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given

volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 1 milligram of cefmenoxime per milliliter (estimated). Transfer 4.0 milliliters of this solution to a 50-milliliter volumetric flask, add 20 milliliters of internal standard solution and dilute to volume with mobile phase to obtain a solution containing 80 micrograms of cefmenoxime per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) for the cefmenoxime peak is satisfactory if it is not more than 1.6 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,200 theoretical plates for the cefmenoxime peak.

(C) *Resolution.* The resolution (*R*) between the peak for cefmenoxime and phthalimide is satisfactory if it is not less than 2.3.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.363(b) of this chapter.

(iv) *Calculations*—(A) *Micrograms per milligram.* Calculate the micrograms of cefmenoxime per milligram as follows:

$$\text{Micrograms of cefmenoxime per milligram} = \frac{\mathrm{T3}R_u \times P_3 \times 100 \times d}{R_s \times C_u(100 - \mathrm{L} - \mathrm{S})}$$

where:

R_u=Area of the cefmenoxime peak in the chromatogram of the sample/Area of internal standard peak;

R_s=Area of the cefmenoxime peak in the chromatogram of the cefmenoxime working standard/Area of internal standard peak;≤

P_s=Cefmenoxime activity in the cefmenoxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution;

d=Dilution factor of the sample;

L=Percent loss on drying (determined as directed in paragraph (b)(4) of this section); and

S=Percent sodium carbonate (determined as directed in paragraph (b)(6) of this section).

(B) *Milligrams of cefmenoxime per vial.* Calculate the cefmenoxime content of the vial as follows:

$$\text{Milligrams of cefmenoxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefmenoxime peak in the chromatogram of the sample/Area of internal standard peak;

R_s=Area of the cefmenoxime peak in the chromatogram of the cefmenoxime working standard/Area of internal standard peak;

P_s=Cefmenoxime activity in the cefmenoxime working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 60 milligrams of cefmenoxime per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Sodium carbonate content.* Proceed as directed in § 436.364 of this chapter.

[53 FR 13403, Apr. 25, 1988; 53 FR 19369, May 27, 1988]

§ 442.223 Sterile cephaloridine.

The requirements for certification and the tests and methods of assay for sterile cephaloridine packaged for dispensing are described in § 442.23a.

[39 FR 19040, May 30, 1974, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.225 Cephalothin sodium injectable dosage forms.

§ 442.225a Sterile sodium cephalothin.

The requirements for certification and the tests and methods of assay for sterile sodium cephalothin packaged for dispensing are described in § 442.25a.

[39 FR 19040, May 30, 1974. Redesignated at 40 FR 11351, Mar. 11, 1975]

§ 442.225b Cephalothin sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalothin sodium injection is a frozen aqueous solution of cephalothin sodium with one or more suitable and harmless buffer substances. It may contain sodium chloride or dextrose. Each milliliter contains cephalothin sodium equivalent to 20 milligrams, 40 milligrams, or 100 milligrams of cephalothin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephalothin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 6.0 and not more than 8.5. The cephalothin sodium used conforms to the standards prescribed by § 442.25a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cephalothin sodium used in making the batch for potency, loss on drying, pH, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The cephalothin sodium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the ampoule contents as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephalothin per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter, preparing the sample as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with distilled water to give a stock solution of convenient concentration. Further dilute with distilled water to the prescribed concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephalothin per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[40 FR 11351, Mar. 11, 1975, as amended at 49 FR 13493, Apr. 5, 1984; 50 FR 19919, May 13, 1985]

§ 442.225c Cephalothin sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalothin sodium for injection is a dry mixture of cephalothin sodium with one or more suitable and harmless buffer substances. The cephalothin sodium may be isolated in the manufacture of cephalothin sodium for injection. Its cephalothin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of cephalothin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 6.0 and not more than

8.5. If isolated, the cephalothin sodium used conforms to the standards prescribed by § 442.25a(a)(1). If the cephalothin sodium is not isolated: The potency of the dry mixture is not less than 850 micrograms of cephalothin per milligram on an anhydrous basis when corrected for sodium bicarbonate; the specific rotation of the dry mixture in an aqueous solution containing 50 milligrams of cephalothin per milliliter at 25° C is +129° ±5°; and the dry mixture gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) If isolated, the cephalothin sodium used in making the batch for potency, loss on drying, pH, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH. In addition, if the cephalothin sodium is not isolated, results of tests and assays on the dry mixture for potency, specific rotation, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: A minimum of 10 immediate containers, unless the cephalothin sodium is not isolated, a minimum of 15 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Content; potency*—(i) *Sample preparation.* Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay or distilled water for the hydroxylamine colorimetric assay to obtain a stock solution of convenient concentration. In addition, if the cephalothin sodium is not isolated, dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay or distilled water for the hydroxylamine colorimetric assay to obtain a stock solution of convenient concentration. Correct the potency, micrograms of cephalothin per milligram, for sodium bicarbonate content determined as described in paragraph (b)(7) of this section.

(ii) *Assay procedures.* Use either of the following methods; however, the results obtained from the hydroxylamine colorimetric assay shall be conclusive.

(*a*) *Microbiological agar diffusion assay.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of cephalothin per milliliter (estimated).

(*b*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of cephalothin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

(7) *Specific rotation.* Dissolve and dilute an accurately weighed portion of the dry mixture with sufficient distilled water to give a concentration of approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis and correct for sodium bicarbonate content. Determine the sodium bicarbonate content as follows: Dissolve an accurately weighed portion of the dry mixture, approximately 1.0 gram, with approximately 50 milliliters of distilled water. Titrate with 0.1*N* sulfuric acid.

Determine the end-point potentiometrically using a glass calomel combination electrode. Each milliliter of 0.1*N* sulfuric acid is equivalent to 8.401 milligrams of sodium bicarbonate.

(8) *Identity*. Using a 0.0025-percent solution of the sample in water and a suitable spectrophotometer, record the ultraviolet absorption spectrum from 220 to 310 nanometers. The spectrum compares qualitatively to that of the working standard similarly tested.

[40 FR 5355, Feb. 5, 1975, as amended at 46 FR 38503, July 28, 1981; 48 FR 51293, Nov. 8, 1983; 49 FR 5097, Feb. 10, 1984; 50 FR 19919, May 13, 1985]

§ 442.229 Sterile cephapirin sodium.

The requirements for certification and the tests and methods of assay for sterile cephapirin sodium packaged for dispensing are described in § 442.29a.

§ 442.240 Cephradine injectable dosage forms.

§ 442.240a Cephradine for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cephradine for injection is a dry mixture of cephradine and one or more suitable and harmless solubilizing and buffering agents. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cephradine that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 8.0 and not more than 9.6. The cephradine used conforms to the standards prescribed by § 442.40a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sterile cephradine used in making the batch for potency, moisture, pH, cephalexin content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The cephradine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Microbiological agar diffusion assay*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling for intramuscular use. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with solution 1 to the reference concentration of 10.0 micrograms of cephradine per milliliter (estimated).

(ii) *Hydroxylamine colorimetric assay*. Proceed as directed in § 442.40(b)(1)(ii), preparing the sample as follows: Reconstitute the sample as directed in the labeling for intramuscular use. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute an aliquot of this solution with distilled water to 1 milligram of cephradine per milliliter (estimated)

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 80 milligrams of cephradine per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

[40 FR 51626, Nov. 6, 1975. Redesignated at 43 FR 14646, Apr. 7, 1978; 50 FR 19919, May 13, 1985]

§ 442.240b Sterile cephradine.

The requirements for certification and the tests and methods of assay for sterile cephradine packaged for dispensing are described in § 442.40a.

[43 FR 14646, Apr. 7, 1978]

§ 442.250 Ceforanide for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Ceforanide for injection is a dry mixture of ceforanide and *L*-lysine. Each milligram of ceforanide for injection contains not less than 900 micrograms and not more than 1,050 micrograms of ceforanide when corrected for *L*-lysine content. Its ceforanide content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of ceforanide that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 8.5. The ceforanide used conforms to the standards prescribed by § 442.50a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sterile ceforanide used in making the batch for ceforanide content, moisture, pH, and identity.

(*b*) The batch for ceforanide content, sterility, pyrogens, moisture, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The ceforanide used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Ceforanide content.* Determine both micrograms of ceforanide per milligram of sample and milligrams of ceforanide per container. Proceed as directed in § 436.348 of this chapter, preparing the sample solution and calculating the ceforanide content as follows:

(i) *Preparation of sample solution.* Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(i) (*a*) and (*b*) of this section.

(*a*) *Micrograms of ceforanide per milligram.* Prepare a solution containing 1.0 milligrams per milliliter in mobile phase. Inject each sample within 5 minutes after dissolution.

(*b*) *Milligrams of ceforanide per container.* Reconstitute the sample with distilled water as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of ceforanide content in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with mobile phase to obtain a stock solution containing 10.0 milligrams per milliliter (estimated). Immediately dilute an aliquot of the stock solution with mobile phase to a concentration of 1.0 milligrams of ceforanide per milliliter (estimated). Inject within 5 minutes, after preparation.

(ii) *Calculations*—(*a*) *Micrograms of ceforanide per milligram.* Calculate the micrograms of ceforanide per milligram of sample as follows:

$$\text{Micrograms of ceforanide per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - L)}$$

where:

A_u=Area of the ceforanide sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the ceforanide peak in the chromatogram of the ceforanide working

standard;

P_s=Ceforanide activity in the ceforanide working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

L=Percent lysine content of the sample. (Determined as described in § 436.349 of this chapter.)

(*b*) *Milligrams of ceforanide per vial.* Calculate the ceforanide content of the vial as follows:

$$\text{Milligrams of ceforanide per vial} = \frac{A_s \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ceforanide sample peak (at a retention time equal to that observed for the standard);

A_s=Area of the ceforanide peak in the chromatogram of the ceforanide working standard;

P_s=Ceforanide activity in the ceforanide working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except reconstitute the vials with approximately 3.0 milliliters of diluting fluid A per each gram of antibiotic activity. Transfer approximately 1 milliliter from each of 20 vials into a sterile 500-milliliter Erlenmeyer flask containing 200 milliliters of diluting fluid A. Filter as described in paragraph (e)(1)(ii) of this section, except in lieu of filtering with three 100-milliliter quantities of diluting fluid A, rinse the filter membrane with three 100-milliliter portions of diluting fluid D followed by a final rinse with 100 milliliters of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of ceforanide per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the product is reconstituted as directed in the labeling.

[49 FR 25848, June 25, 1984; 49 FR 34347, Aug. 30, 1984; 49 FR 40006, Oct. 12, 1984, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.253 Cefotetan injectable dosage forms.

§ 442.253a Sterile cefotetan disodium.

The requirements for certification and the tests and methods of assay for sterile cefotetan disodium packaged for dispensing are described in § 442.53a.

[51 FR 20264, June 4, 1986. Redesignated at 59 FR 26941, May 25, 1994]

§ 442.253b Cefotetan sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotetan sodium injection is a frozen, aqueous, iso-osmotic solution of cefotetan and sodium bicarbonate. It contains one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains cefotetan disodium equivalent to 20 milligrams or 40 milligrams of cefotetan per milliliter. Its cefotetan content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefotetan that it is represented to contain. It is sterile. It contains not more than 0.17 endotoxin units per milligram of cefotetan. Its pH is not less than 4.0 and not more than 6.5. It passes the identity test. The cefotetan used conforms to the standards prescribed by § 442.52(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefotetan used in making the batch for cefotetan potency, moisture, and identity.

(B) The batch for cefotetan potency, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefotetan used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*. Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Cefotetan potency*. Proceed as directed in §442.52(b)(1), except prepare the sample solution and calculate the cefotetan content as follows:

(i) *Preparation of sample solution*. Using a suitable hypodermic needle and syringe, remove an accurately measured portion from each container immediately after thawing and reaching room temperature and dilute with mobile phase to obtain a solution containing 200 micrograms of cefotetan per milliliter (estimated). Prepare the sample solution just prior to its introduction into the chromatograph.

(ii) *Calculation*. Calculate the milligrams of cefotetan per milliliter of sample as follows:

$$\text{Micrograms of cefotetan per milligram} = \frac{A_U \times P_s \times 100}{A_s \times C_U \times (100 - m)}$$

where:

A_U=Area of the cefotetan peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the cefotetan peak in the chromatogram of the cefotetan working standard;

P_S=Cefotetan activity in the cefotetan working standard solution in micrograms per milliliter;

C_U=Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Bacterial endotoxins*. Proceed as directed in the U.S. Pharmacopeia bacterial endotoxins test.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefotetan working standard.

[59 FR 26941, May 25, 1994]

§442.255 Ceftriaxone injectable dosage forms.

§442.255a Sterile ceftriaxone sodium.

The requirements for certification and the tests and methods of assay for sterile ceftriaxone sodium packaged for dispensing as described in §442.55a.

[50 FR 10001, Mar. 13, 1985. Redesignated at 52 FR 44860, Nov. 23, 1987]

§442.255b Ceftriaxone sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Ceftriaxone sodium injection is a frozen aqueous iso-osmotic solution of ceftriaxone sodium which may contain one or more suitable and harmless buffer substances. Each milliliter contains ceftriaxone sodium equivalent to 10, 20, or 40 milligrams of ceftriaxone per milliliter. Its ceftriaxone content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of ceftriaxone that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 6.0 and not more than 8.0. It passes the identity test. The ceftriaxone sodium used conforms to the standards prescribed by §442.55(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The ceftriaxone sodium used in making the batch for potency, moisture, pH, crystallinity, and identity.

(B) The batch for content, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The ceftriaxone sodium used in making the batch: 10 packages, each containing 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Ceftriaxone content.* Proceed as directed in § 442.55a(b)(1) of this chapter, except prepare the sample solution and calculate the ceftriaxone content as follows:

(i) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature and dilute with mobile phase to obtain a solution containing 180 micrograms of ceftriaxone per milliliter (estimated). Prepare the sample solution just prior to its introduction into the chromatograph.

(ii) *Calculation.* Calculate the milligrams of ceftriaxone anhydrous free acid per milliliter of sample as follows:

$$\text{Milligrams of ceftriaxone anhydrous free acid per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the ceftriaxone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the ceftriaxone peak in the chromatogram of the ceftriaxone working standard;

P_s=Ceftriaxone activity in the ceftriaxone working standard solution in micrograms of anhydrous free acid per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 40 milligrams of ceftriaxone per kilogram.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identify.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the ceftriaxone working standard.

[52 FR 44860, Nov. 23, 1987, as amended at 55 FR 11583, Mar. 29, 1990]

§ 442.258 Cefotiam dihydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefotiam dehydrochloride for injection is a dry mixture of cefotiam dihydrochloride and sodium carbonate. Its cefotiam potency is satisfactory if each milligram of cefotiam dihydrochloride for injection contains not less than 790 micrograms and not more than 925 micrograms of cefotiam on an anhydrous basis, when corrected for sodium carbonate content. Its cefotiam content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefotiam that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 6.0 percent. The pH of an aqueous solution containing 100 milligrams per milliliter is not less than 5.7 and not more than 7.2. The cefotiam dihydrochloride used conforms to the standards prescribed by § 442.58a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefotiam dihydrochloride used in making the batch for potency, moisture, identity, and crystallinity.

(B) The batch for cefotiam potency, cefotiam content, sterility, pyrogens, loss on drying, pH, and sodium carbonate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefotiam dihydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefotiam potency and content.* Determine both micrograms of cefotiam per milligram of sample and milligrams of cefotiam per container. Proceed as directed in § 442.58a(b)(1), preparing the sample solutions and calculating the potency and content as follows:

(i) *Preparation of sample solutions.* Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(i) (A) and (B) of this section.

(A) *Cefotiam potency (micrograms of cefotiam per milligram).* Dissolve an accurately weighed sample with sufficient distilled water to obtain a solution containing approximately 1,000 micrograms of cefotiam per milliliter. Further dilute this solution with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter (estimated).

(B) *Cefotiam content (milligrams of cefotiam per vial).* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 1,000 micrograms of cefotiam activity per milliliter (estimated). Further dilute this solution with mobile phase to obtain a solution containing 50 micrograms of cefotiam activity per milliliter (estimated).

(ii) *Calculations*—(A) *Cefotiam potency (micrograms per milligram).* Calculate the micrograms of cefotiam per milligram as follows:

$$\text{Micrograms of cefotiam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - L - S)}$$

where:

A_u=Area of the cefotiam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotiam peak in the chromatogram of the cefotiam working standard;

P_s=Cefotiam activity in the cefotiam working standard solution in micrograms per milliliter;

C_u=Milligrams of the sample per milliliter of sample solution;

L=Percent loss on drying (determined as directed in paragraph (b)(4) of this section); and

S=Percent sodium carbonate (determined as directed in paragraph (b)(6) of this section).

(B) *Cefotiam content (milligrams of cefotiam per vial).* Calculate the cefotiam content of the vial as follows:

$$\text{Milligrams of cefotiam per vial} = \frac{A_u \times P_s \times d}{A^s \times 1{,}000}$$

where:

A_u=Area of the cefotiam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefotiam peak in the chromatogram of the cefotiam working standard;

P_s=Cefotiam activity in the cefotiam working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(g) of this chapter, using a solution containing 40 milligrams of cefotiam per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Sodium carbonate content.* Proceed as directed in § 436.357 of this chapter.

[54 FR 20786, May 15, 1989]

§ 442.260 Cefpiramide sodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cefpiramide sodium for injection is a dry mixture of cefpiramide and sodium benzoate. It contains other buffers and preservatives. Its cefpiramide potency is satisfactory if each milligram of cefpiramide sodium for injection contains not less than 754 micrograms and not more than 924

micrograms of cefpiramide on an anhydrous basis. Its cefpiramide content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of cefpiramide that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 6.0 and not more than 8.0. It passes the identity test. The cefpiramide used conforms to the standards prescribed by § 442.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefpiramide used in making the batch for potency, moisture, pH, total related substances, specific rotation, identity, and crystallinity.

(B) The batch for cefpiramide potency, cefpiramide content, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The cefpiramide used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cefpiramide potency and content.* Determine both micrograms of cefpiramide per milligram of sample and milligrams of cefpiramide per container. Proceed as directed in § 442.60(b)(1), preparing the sample solutions and calculating the potency and content as follows:

(i) *Preparation of sample solutions.* Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(i)(A) and (b)(1)(i)(B) of this section.

(A) *Cefpiramide potency (micrograms of cefpiramide per milligram*). Dissolve an accurately weighed sample with sufficient mobile phase to obtain a solution containing approximately 0.25 milligram of cefpiramide per milliliter (estimated).

(B) *Cefpiramide content (milligrams of cefpiramide per vial*). Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient distilled water to obtain a solution containing 1.0 milligram of cefpiramide activity per milliliter (estimated). Further dilute this solution with mobile phase to obtain a solution containing 0.25 milligram of cefpiramide activity per milliliter (estimated).

(ii) *Calculations*—(A) *Cefpiramide potency (micrograms per milligram*). Calculate the micrograms of cefpiramide per milligram as follows:

$$\text{Micrograms of cefpiramide per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cefpiramide peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cefpiramide peak in the chromatogram of the cefpiramide working standard;

P_s=Cefpiramide activity in the cefpiramide working standard solution in micrograms per milliliter;

C_u=Milligrams of the sample per milliliter of sample solution;

m =Percent moisture content of the sample.

(B) *Cefpiramide content (milligrams of cefpiramide per vial).* Calculate the cefpiramide content of the vial as follows:

$$\text{Milligrams of cefpiramide per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cefpiramide peak in the chromatogram of the sample (a ta retention time equal to that observed for the standards);

A_s=Area of the cefpiramide peak in the chromatogram of the cefpiramide working standard;

P_s=Cefpiramide activity in the cefpiramide working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in §436.20(e)(1).

(3) *Pyrogens*. Proceed as directed in §436.32(b) of this chapter, using a solution containing 50 milligrams of cefpiramide per milliliter.

(4) *Moisture*. Proceed as directed in §436.201 of this chapter.

(5) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Identify*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefpiramide working standard.

[55 FR 14242, Apr. 17, 1990]

§442.270 Cefmetazole injectable dosage forms.

§442.270a Sterile cefmetazole sodium.

The requirements for certification and the tests and methods of assay for sterile cefmetazole sodium packaged for dispensing are described in §442.70a.

[55 FR 6636, Feb. 26, 1990]

§442.270b Cefmetazole sodium injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Cefmetazole sodium injection is a frozen, aqueous, iso-osmotic solution of cefmetazole and sodium citrate. It contains one or more suitable and harmless buffer substances and a tonicity adjusting agent. Each milliliter contains cefmetazole sodium equivalent to 20 milligrams or 40 milligrams of cefmetazole per milliliter. Its cefmetazole content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cefmetazole that it is represented to contain. It is sterile. It contains not more than 0.2 endotoxin units per milligram. Its pH is not less than 4.2 and not more than 6.2. It passes the identity test. The cefmetazole used conforms to the standards prescribed by §442.69(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cefmetazole used in making the batch for potency, moisture, and identity.

(B) The batch for potency, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The cefmetazole used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*. Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Cefmetazole potency*. Proceed as directed in §442.70a(b)(1), except prepare the sample solution and calculate the cefmetazole content as follows:

(i) *Preparation of sample solution*. Using a suitable hypodermic needle and syringe, remove an accurately measured portion from each container immediately after thawing and reaching room temperature and dilute with mobile phase to obtain a solution containing 500 micrograms of cefmetazole per milliliter (estimated). Prepare the sample solution just prior to its introduction into the chromatograph.

(ii) *Calculation*. Calculate the milligrams of cefmetazole per milliliter of sample as follows:

$$\text{Milligrams of cefmetazole per milliliter} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_U=Area of the cefmetazole peak in the chromatogram of the – sample (at a retention time equal to that observed for the standard);

A_S=Area of the cefmetazole peak in the chromatogram of the cefmetazole working standard;

P_S=Cefmetazole activity in the cefmetazole working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Bacterial endotoxins*. Proceed as directed in the United States Pharmacopeia bacterial endotoxins test.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the cefmetazole working standard.

[59 FR 12546, Mar. 17, 1994]

PART 443—CARBACEPHEM ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
443.20 Loracarbef.

Subpart B—Oral Dosage Forms

443.120 Loracarbef oral dosage forms.
443.120a Loracarbef capsules.
443.120b Loracarbef for oral suspension.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 58 FR 26667, May 4, 1993, unless otherwise noted.

Subpart A—Bulk Drugs

§443.20 Loracarbef.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Loracarbef is the monohydrate form of (6*R*,7*S*)-7-[(*R*)-2-amino-2-phenylacetamido]-3-chloro-8-oxo-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. It is so purified and dried that:

(i) Its potency is not less than 960 micrograms and not more than 1,020 micrograms of loracarbef activity per milligram, on an anhydrous basis.

(ii) Its moisture content is not less than 3.5 percent and not more than 6.0 percent.

(iii) The pH of an aqueous slurry containing 100 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(iv) Its specific rotation in an 0.1 *N* HCl solution containing 10 milligrams of loracarbef per milliliter at 25° C is +27°to+33° on an anhydrous basis.

(v) It is crystalline.

(vi) It gives a positive identity test.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for loracarbef potency, moisture, pH, specific rotation, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 265 nanometers, a 25-centimeter by 4.6-millimeter (inside diameter) column packed with microparticulate (5 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded to silicas, a flow rate of 1.5 milliliters per minute, and a known injection volume between 10 and 20 microliters. The retention time for loracarbef is between 10 and 13 minutes. Mobile phase, working standard, sample and resolution test solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Dissolve 2.0 grams of pentanesulfonic acid sodium salt monohydrate in 1,560 milliliters of water. Add 20 milliliters of triethylamine. Adjust the pH to 2.5 with phosphoric acid. Add 440 milliliters of methanol and mix. Filter the mobile phase through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution*. Accurately weigh approximately 10 milligrams of the loracarbef working reference standard into a 50-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase. Brief sonication may be required to obtain complete dissolution of the material.

(B) *Sample solution*. Accurately weigh approximately 10 milligrams of sample into a 50-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase. Brief sonication may be required to obtain complete dissolution of the material.

(C) *Resolution test solution*. Prepare a resolution test solution containing approximately 0.2 milligram per milliliter each of loracarbef and loracarbef *L*-isomer in the mobile phase.

(iii) *System suitability requirements*—(A) *Asymmetry factor*. The asymmetry factor (A_S) at 5 percent peak height is satisfactory if it is not less than 0.8 and not more than 1.3 for the loracarbef peak.

(B) *Efficiency of the column*. The absolute efficiency (h_r) is satisfactory if it is not more than 20 for the loracarbef peak.

(C) *Resolution factor*. The resolution factor (R) between the peak for loracarbef and the peak for the resolution standard loracarbef *L*-isomer in the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′)*. The capacity factor (k') for loracarbef is satisfactory if it is not less than 5 and not more than 8.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of loracarbef per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of loracarbef per milligram} = \frac{A_U \times P_s \times 100}{A_s \times C_U \times (100 - m)}$$

where:

A_U=Area of the loracarbef peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the loracarbef peak in the chromatogram of the loracarbef working standard;

P_S=Loracarbef activity in the loracarbef working standard solution in micrograms per milliliter;

C_U=Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 100 milligrams per milliliter.

(4) *Specific rotation*. Dissolve and dilute an accurately weighed sample with sufficient 0.1 *N* HCl to obtain a concentration of approximately 10 milligrams of loracarbef activity per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(5) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the 1.0 percent potassium bromide disc prepared as described in § 436.211(b)(1).

Subpart B—Oral Dosage Forms

§ 443.120 Loracarbef oral dosage forms.

§ 443.120a Loracarbef capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Loracarbef capsules are composed of loracarbef and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains loracarbef equivalent to either 200 milligrams or 400 milligrams of loracarbef. Its loracarbef content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of loracarbef that it is represented to contain. Its moisture content is not more than 8.5 percent. It passes the dissolution test. It passes the identity test. The loracarbef used

conforms to the standards prescribed by § 443.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The loracarbef used in making the batch for potency, moisture, pH, specific rotation, crystallinity, and identity.

(B) The batch for content, moisture, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The loracarbef used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Loracarbef content.* Proceed as directed in § 443.20(b)(1), preparing the sample solution and calculating the loracarbef content as follows:

(i) *Preparation of sample solution.* Place one intact capsule in a 200-milliliter volumetric flask containing 150 milliliters of distilled water. Shake the mixture vigorously to aid disruption of the capsule. Sonicate the mixture briefly (5 minutes). Dilute the contents to volume with distilled water. Mix well and immediately transfer a suitable aliquot to a volumetric flask of appropriate size to obtain a solution containing 0.2 milligram per milliliter (estimated) of loracarbef when diluted to volume with mobile phase (described in § 443.20(b)(1)(i)). Filter this solution through a 0.45-micron membrane filter before injecting it into the chromatograph.

(ii) *Calculations.* Calculate the loracarbef content as follows:

$$\text{Milligrams of loracarbef per capsule} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_U=Area of the loracarbef peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the loracarbef peak in the chromatogram of the loracarbef working standard;

P_S=Loracarbef activity in the loracarbef working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity Q, the amount of loracarbef activity dissolved, is 75 percent within 30 minutes.

(4) *Identity.* The retention time of the loracarbef response in the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section as applied to the sample solution compares qualitatively to that of the loracarbef reference standard.

§ 443.120b Loracarbef for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Loracarbef for oral suspension is loracarbef with one or more suitable and harmless preservatives, sweeteners, suspending agents, colorings, antifoaming agents, and flavorings. When constituted as directed in the labeling, each milliliter contains the equivalent of either 20 or 40 milligrams loracarbef activity. Its loracarbef content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of loracarbef that it is represented to contain. Its moisture content is not more than 2.0 percent. When constituted as described in the labeling, the pH of the suspension is not less than 3.5 and not more than 6.0. It passes the identity test. The loracarbef used conforms to the standards prescribed by § 443.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The loracarbef used in making the batch for potency, moisture, pH, specific rotation, crystallinity, and identity.

(B) The batch for content, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The loracarbef used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Loracarbef content*. Proceed as directed in §443.20(b)(1), preparing the sample solution and calculating the loracarbef content as follows:

(i) *Preparation of sample solution*. Constitute as directed in the labeling. Transfer a 5.0-milliliter portion of the suspension into an appropriately sized volumetric flask and quantitatively dilute stepwise with mobile phase (described in §443.20(b)(1)(i)) to obtain a concentration of 0.2 milligram of loracarbef activity per milliliter (estimated).

(ii) *Calculations*. Calculate the loracarbef content as follows:

$$\text{Milligrams of loracarbef per 5 milliliters of sample} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_U=Area of the loracarbef peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the loracarbef peak in the chromatogram of the loracarbef working standard;

P_S=Loracarbef activity in the loracarbef working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using the drug constituted as directed in the labeling.

(4) *Identity*. The retention time of the loracarbef response in the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section as applied to the sample solution compares qualitatively to that of the loracarbef reference standard.

PART 444—OLIGOSACCHARIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
444.6 Amikacin.
444.7 Amikacin sulfate.
444.10a Dihydrostreptomycin sulfate, crystalline dihydrostreptomycin sulfate, dihydrostreptomycin hydrochloride.
444.20 Gentamicin sulfate.
444.20a Sterile gentamicin sulfate.
444.30 Kanamycin sulfate.
444.30a Sterile kanamycin sulfate.
444.42 Neomycin sulfate.
444.42a Sterile neomycin sulfate.
444.46 Netilmicin sulfate.
444.50 Paromomycin sulfate.
444.62 Sisomicin sulfate.
444.70a Sterile streptomycin sulfate.
444.80 Tobramycin.
444.81a Sterile tobramycin sulfate.

Subpart B—Oral Dosage Forms

444.130 Kanamycin sulfate capsules.
444.142 Neomycin sulfate oral dosage forms.
444.142a Neomycin sulfate tablets.
444.142b Neomycin sulfate oral solution.
444.150 Paromomycin sulfate oral dosage forms.
444.150a Paromomycin sulfate capsules.
444.150b Paromomycin sulfate sirup.

Subpart C—Injectable Dosage Forms

444.206 Amikacin sulfate injection.
444.220 Gentamicin sulfate injection.
444.230 Kanamycin sulfate injection.
444.246 Netilmicin sulfate injection.
444.262 Sisomicin sulfate injection.
444.270 Streptomycin sulfate injectable dosage forms.
444.270a Sterile streptomycin sulfate.
444.270b Streptomycin sulfate injection.
444.280 Tobramycin sulfate injection.
444.281 Sterile tobramycin sulfate.

Subpart D—Ophthalmic Dosage Forms

444.320 Gentamicin sulfate ophthalmic dosage forms.
444.320a Gentamicin sulfate ophthalmic solution.
444.320b Gentamicin sulfate ophthalmic ointment.
444.320c Gentamicin sulfate-prednisolone acetate ophthalmic suspension.
444.320d Gentamicin sulfate-prednisolone acetate ophthalmic ointment.
444.342 Neomycin sulfate ophthalmic dosage forms.
444.342a Neomycin sulfate- ———— ophthalmic suspension; neomycin sulfate- ———— ophthalmic solution (the blanks being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).
444.342b Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.
444.342c Neomycin sulfate- gramicidin

———— ophthalmic solution; neomycin sulfate-gramicidin ———— ophthalmic suspension (the blanks being filled in with the established name(s) of the other ingredient(s) present in accordance with paragraph (a)(1) of this section).

444.342d Neomycin sulfate-polymyxin B sulfate ———— ophthalmic suspension (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

444.342e Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

444.342f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

444.342g Neomycin sulfate-hydrocortisone acetate ophthalmic suspension; neomycin sulfate-prednisolone acetate ophthalmic suspension.

444.342h Neomycin sulfate-polymyxin B sulfate ophthalmic ointment.

444.342i Neomycin sulfate-polymyxin B sulfate ophthalmic solution.

444.342j Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic suspension.

444.342k Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic ointment.

444.380 Tobramycin ophthalmic dosage forms.

444.380a Tobramycin ophthalmic solution.

444.380b Tobramycin ophthalmic ointment.

444.380c Tobramycin–dexamethasone ophthalmic suspension.

444.380d Tobramycin–dexamethasone ophthalmic ointment.

444.380e Tobramycin–fluorometholone acetate ophthalmic suspension.

Subpart E—Otic Dosage Forms

444.442 Neomycin sulfate otic dosage forms.

444.442a—444.442c [Reserved]

444.442d Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

444.442e [Reserved]

444.442f Neomycin sulfate-hydrocortisone-acetic acid otic suspension.

444.442g Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension.

444.442h Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic solution.

Subpart F—Dermatologic Dosage Forms

444.520 Gentamicin sulfate dermatologic dosage forms.

444.520a Gentamicin sulfate ointment.

444.520b Gentamicin sulfate cream.

444.540 Neomycin palmitate dermatologic dosage forms.

444.542 Neomycin sulfate dermatologic dosage forms.

444.542a Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

444.542b Neomycin sulfate cream; neomycin sulfate_____ cream (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

444.542c Neomycin sulfate- ———— lotion (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

444.542d [Reserved]

444.542e Neomycin sulfate-polymyxin B sulfate ointment.

444.542f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

444.542g Neomycin sulfate-gramicidin-triamcinolone acetonide cream.

444.542h Neomycin sulfate-gramicidin-triamcinolone acetonide lotion; neomycin sulfate-gramicidin-fludrocortisone acetate lotion.

444.542i [Reserved]

444.542j Neomycin sulfate-polymyxin B sulfate-gramicidin-benzocaine ointment.

444.542k Neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate cream.

444.542l Neomycin sulfate-polymyxin B sulfate cream.

Subparts G–I [Reserved]

Subpart J—Certain Other Dosage Forms

444.942 Neomycin sulfate in certain other dosage forms.

444.942a Neomycin sulfate for compounding oral products.

444.942b Sterile neomycin sulfate and polymyxin B sulfate solution.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19046, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§444.6 Amikacin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amikacin is A-3-amino-3-deoxy-A-D-glucopyranosyl (1–6) - A - [6 - amino - 6 - deoxy - A - D - glucopyranosyl (1–4)] - N^1 - [(*s*) - 4 - amino - 2 - hydroxy - 1 - oxobutyl] - 2 - deoxy - D - streptamine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 8.5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 9.5 and not more than 11.5.

(v) It gives a positive identity test for amikacin.

(vi) Its residue on ignition is not more than 1.0 percent.

(vii) Its specific rotation is not less than +97° and not more than +105°.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, identity, residue on ignition, specific rotation, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 10.0 micrograms of amikacin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* Proceed as directed in §436.318 of this chapter.

(6) *Residue on ignition.* Proceed as directed in §436.207(a) of this chapter.

(7) *Specific rotation.* Proceed as directed in §436.210 of this chapter, using an aqueous solution containing 20 milligrams of amikacin per milliliter and a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[41 FR 49483, Nov. 9, 1976, as amended at 44 FR 10379, Feb. 20, 1979; 50 FR 19919, May 13, 1985]

§444.7 Amikacin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amikacin sulfate is the sulfate salt of *D*-streptamine, *0*-3-amino-3-deoxy-α-*D*-glucopyranosyl(1-6)-*0*-[6-amino-6-deoxy-α-*D*-glucopyranosyl(1-4)]-N^1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-,(S)-. It is so purified and dried that:

(i) Its potency is not less than 674 micrograms and not more than 786 micrograms per milligram on an anhydrous basis if the molar ratio of amikacin to sulfuric acid (H_2SO_4) is 1:2 and is not less than 691 micrograms and not more than 806 micrograms per milligram on an anhydrous basis if the molar ratio of amikacin to H_2SO_4 is 1:1.8.

(ii) Its loss on drying is not more than 13.0 percent.

(iii) The pH of an aqueous solution containing 10 milligrams of amikacin sulfate per milliliter is not less than 2.0 and not more than 4.0 if the molar ratio of amikacin to H_2SO_4 is 1:2 and not less than 6.0 and not more than 7.3 if the molar ratio of amikacin to H_2SO_4 is 1:1.8.

(iv) It gives a positive identify test for amikacin.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) Its specific rotation is not less than +76° and not more than +84° on the anhydrous basis.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, identity, residue on ignition, specific rotation, and crystallinity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using a 25-centimeter by 4.6-millimeter column packed with irregular 5-micron octadecyl hydrocarbon bonded silica, thermostatted at 30°C, an ultraviolet detection system operating at a wavelength of 340 nanometers, a flow rate not exceeding 2.0 milliliters per minute, a chart speed of 1.0 centimeter per minute (the chart speed is increased to 5.0 centimeters per minute to obtain chromatograms used for performance parameter determinations), and a known injection volume between 15.0 and 30.0 microliters. Retention times of amikacin and kanamycin are about 10 and 15 minutes, respectively. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0 percent 2,4,6-trinitrobenzenesulphonic acid solution.* Dissolve 1.0 gram of 2,4,6-trinitrobenzenesulphonic acid in 100 milliliters of distilled water.

(B) *0.02M potassium dihydrogen phosphate.* Dissolve 2.72 grams of potassium dihydrogen phosphate in 800 milliliters of distilled water and mix to dissolve the solid. Dilute to 1,000 milliliters with distilled water and mix.

(C) *Mobile phase.* Mix 0.02*M* potassium dihydrogen phosphate and methanol, high performance liquid chromatography reagent grade (28:72 by volume). Adjust the pH to 6.5 with 0.4*M* potassium hydroxide. Filter the mobile phase through a suitable glass filter or equivalent which is capable of removing particulate matter contamination greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions.* (A) *Working standard solution.* Dissolve an accurately weighed portion of the amikacin working standard with sufficient distilled water to obtain a solution containing approximately 1.0 milligram of amikacin activity per milliliter. This preparation is stable for 1 week. Transfer 50 microliters of this solution directly to the bottom of a 50-milliliter, glass-stoppered centrifuge tube, using an automatic micropipetter. Add 3.2 milliliters of pyridine and 2.0 milliliters of 1 percent 2,4,6-trinitrobenzenesulphonic acid reagent just above the surface of the solution in the centrifuge tube. Close the tube tightly, mix and heat the tube in a water bath maintained at 75°C±1° for 45 minutes. Remove the tube from the bath and cool it at room temperature. Filter the contents through a 0.5 micron membrane. Use the filtrate for the quantitative chromatographic determinations.

(B) *Preparation of sample solution.* Dissolve an accurately weighed portion of sample with sufficient distilled water to obtain a solution containing 1.0 milligram of amikacin activity per milliliter (estimated). This preparation is stable for 1 week. Proceed as directed in paragraph (b)(1)(ii)(A) of this section, beginning at ''Transfer 50 microliters * * *''.

(C) *Resolution test solution.* Prepare an aqueous solution containing about 1.0 milligram per milliliter each of amikacin and kanamycin. Proceed as directed in paragraph (b)(1)(ii)(A) of this section, beginning at ''Transfer 50 microliters * * *''.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_s) of the amikacin peak is satisfactory if it is not more than 1.3 at 10 percent of peak height.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 20.0 for the amikacin peak.

(C) *Resolution.* The resolution (R) between the amikacin peak and the kanamycin peak is satisfactory if it is not less than 5.0.

(D) *Coefficent of variation* (relative standard deviation). The coefficient of variation (S_r in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of amikacin per milligram of sample as follows:

$$\text{Micrograms of amikacin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the amikacin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the amikcacin peak in the chromatogram of the amikacin working standard;

P_s=Amikacin activity in the amikacin working standard solution in micrograms per milliliter;

C_u=Milligrams of the sample per milliliter of sample solution; and

m=Percent loss on drying of the sample.

(2) *Loss on drying*. Proceed as directed in § 436.200(c) of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(4) *Identity*. Proceed as directed in § 436.318 of this chapter.

(5) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(6) *Specific rotation*. Proceed as directed in § 436.210 of this chapter, using an aqueous solution containing 20 milligrams of amikacin sulfate per milliliter, and a 1.0 decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[55 FR 38676, Sept. 20, 1990]

§ 444.10a Dihydrostreptomycin sulfate, crystalline dihydrostreptomycin sulfate, dihydrostreptomycin hydrochloride.

(a) *Requirements for certification*—(1) Dihydrostreptomycin sulfate is the hydrogenated sulfate salt of a kind of streptomycin or a mixture of two or more such salts; crystalline dihydrostreptomycin sulfate is the hydrogenated crystalline sulfate salt of a kind of streptomycin or a mixture of two or more such salts; dihydrostreptomycin hydrochloride is the hydrogenated hydrochloride salt of a kind of streptomycin or a mixture of two or more such salts. Each such drug conforms to all requirements prescribed by § 444.70a(a) for streptomycin sulfate and streptomycin hydrochloride, and is subject to all procedures prescribed by § 444.70a(a) for streptomycin sulfate and streptomycin hydrochloride, except that:

(i) Its potency is not less than 650 micrograms per milligram, except that if it is crystalline dihydrostreptomycin sulfate its potency is not less than 725 micrograms per milligram.

(ii) Its content of streptomycin sulfate or streptomycin hydrochloride is not more than 3.0 percent when calculated as streptomycin base, except that if it is crystalline dihydrostreptomycin sulfate its content of streptomycin sulfate is not more than 1.0 percent.

(iii) Its labeling shall conform to the requirements of § 444.70a(a)(3)(iii).

(b) *Tests and methods of assay*—(1) *Potency*. Using the dihydrostreptomycin working standard as a standard of comparison, proceed as directed in § 444.70a(b)(1). Its potency is satisfactory if it contains not less than 90 percent of the number of milligrams that it is represented to contain.

(2) *Content of streptomycin sulfate or streptomycin hydrochloride*—(i) *Reagents*. (*a*) 10 percent ferric chloride stock solution. Dissolve 5 grams of $FeCl_3 \bullet 6H_2O$ in 50 milliliters 0.1*N* HCl.

(*b*) 0.25 percent ferric chloride solution. Dilute 2.5 milliliters of 10 percent ferric chloride in 0.1*N* HCl to 100 milliliters with 0.01*N* MCl. Prepare the solution fresh daily.

(ii) *Standard curve*. Keep the working standard (obtained from the Food and Drug Administration) at −20° C. in tightly stoppered containers which in turn are kept in larger stoppered vials containing a suitable desiccant. Dry an appropriate amount of the working standard at 100° C. and a pressure of 5 millimeters or less for 4 hours. Prepare a stock aqueous solution containing 1.0 milligram of streptomycin base per milliliter. Store this standard solution

in a refrigerator and use for no longer than 2 weeks. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 milliliters of this standard solution and 10 milliliters of distilled water to each of six 25-milliliter volumetric flasks. Add 9.0, 8.0, 7.0, 6.0, and 5.0 milliliters of distilled water to the five tubes, respectively, to give each a total volume of 10 milliliters. To each add 2.0 milliliters of 1*N* NaOH and then heat the flasks in a boiling water bath for 10 minutes. Cool the flasks in ice water for 3 minutes and acidify the solutions with 2.0 milliliters of 1.2*N* HCl. To each flask add 5.0 milliliters of 0.25 percent ferric chloride reagent, make to volume with distilled water, and mix thoroughly. Transfer the colored solutions to 2.0-centimeter absorption cells and measure the percent light transmission at 530 mμ in a suitable photoelectric colorimeter. Set the colorimeter at 100 percent light transmission for the zero concentration and then obtain the percent light transmission of the sample. Prepare a standard curve on semilog paper, plotting the percent light transmission on the logarithmic ordinate scale and the concentration of streptomycin base on the abscissa.

(iii) *Procedure.* Dilute the contents of a vial or a sufficient amount of bulk material to give a concentration of approximately 20 milligrams per milliliter. From the amount of streptomycin obtained, calculate the percent streptomycin as follows:

Percent streptomycin=(Milligrams of streptomycin×100)/(Milligrams of dihydrostreptomycin found in the sample used)

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Toxicity, pyrogens, histamine, moisture, pH, crystallinity.* Proceed as directed in §§ 444.70a(b) (3), (4), (5), (6) and 440.80a(b)(5)(iii) of this chapter.

§ 444.20 Gentamicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate is the sulfate salt of a kind of gentamicin or a mixture of two or more such salts. It is a powder, white to buff in color. It is readily soluble in water but insoluble in ethanol. It is so purified and dried that:

(i) Its potency is not less than 590 micrograms of gentamicin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 18.0 percent.

(iv) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(v) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25°C. is not less than +107° and not more than +121°.

(vi) Its content of gentamicin C_1 is not less than 25 nor more than 50 percent; of gentamicin C_{1a}, not less than 15 nor more than 40 percent; and of gentamicin C_2, not less than 20 nor more than 50 percent.

(vii) It gives a positive identity test for gentamicin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(ii) *Samples required.* 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(*c*) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams of gentamicin per milliliter.

(5) *Specific rotation*. Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(6) *Content of gentamicins* C_1, C_{1a}, and C_2. Proceed as directed in § 444.20a(b)(8).

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.20a Sterile gentamicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile gentamicin sulfate is the sulfate salt of a kind of gentamicin or a mixture of two or more such salts. It is a powder, white to buff in color. It is readily soluble in water but insoluble in ethanol. It is so purified and dried that:

(i) Its potency is not less than 590 micrograms of gentamicin per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 18.0 percent.

(vi) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(vii) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25° C. is not less than +107° and not more than +121°.

(viii) Its content of gentamicin C_1 is not less than 25 nor more than 50 percent; of gentamicin C_{1a}, not less than 15 nor more than 40 percent; and of gentamicin C_2, not less than 20 nor more than 50 percent.

(ix) It gives a positive identity test for gentamicin sulfate.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with sufficient solution 3 to give a reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10.0 milligrams of gentamicin per milliliter.

(5) *Loss on drying*. Proceed as directed in § 436.200(c) of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams of gentamicin per milliliter.

(7) *Specific rotation*. Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(8) *Content of gentamicins* C_1, C_{1a}, and C_2–(i) *Equipment*–(*a*) *Chamber* (*chromatographic*). Use a suitable chromatography jar with a tightly fitting, ground glass contact top for descending chromatography.

(*b*) *Sheets* (*chromatographic*). Cut a 57 × 46-centimeter sheet of Whatman No. 2

filter paper, or chromatographic paper that will produce similar results, into four strips of about 14.25 × 46 centimeters. Draw a starting line 9 centimeters from one end and mark two dots on this line, each 4 centimeters from each edge.

(ii) *Reagents.* Use reagent grade solvents and chemicals.

(iii) *Solvent system.* In each of two separators, equilibrate 200 milliliters of chloroform and 100 milliliters of methanol with 100 milliliters of 17 percent (9 molar) ammonium hydroxide. Without allowing the phases of one to separate, add the entire mixture to the chromatography jar and allow 24 hours for saturation. Allow the second separator to stand until the phases separate and use the lower phase only as the chromatographic solvent.

(iv) *Ninhydrin reagent.* To 1 gram of ninhydrin and 0.1 gram of cadmium acetate, add 3 milliliters of water and 1.5 milliliters of gaacial acetic acid and shake. Add 100 milliliters of *n*-propanol and shake until solution is complete. Keep this solution in a brown bottle under refrigeration.

(v) *Procedure.* Prepare an aqueous solution containing 40 milligrams of the sample per milliliter. Apply 5 microliters of this solution to each dot on the sheet. Prepare two such sheets and place them in the tank so that elution will take place from separate troughs. Fill the two troughs with the chromatographic solvent. Develop the sheets in a descending manner until the solvent front reaches the bottom of the paper (approximately 3½ hours at 25° C.). Remove the sheets and dry in a hood for 30 minutes. Cut each sheet in half, lengthwise. Spray one half with ninhydrin reagent and place the sprayed strip in a drying oven at 100° C. for 1 minute. The gentamicin fractions appear as reddish zones. The zone furthest from the origin is gentamicin C_1, the one closest is gentamicin $C_{1a,}$ and the middle zone is gentamicin C_2. Cut the corresponding zones out of the other unsprayed half of the sheet. Cut each portion of the sheet thus obtianed into small strips and put those from each zone into a separate 125-milliliter glass-stoppered flask. Add 50 milliliters of 0.1*M* potassium phosphate buffer, pH 8, to each flask and swirl the flask mechanically for 30 minutes. Decant the solution from each flask into separate test tubes and allow the paper to settle. Pipet 4 milliliters of each clear solution into a 25-milliliter volumetric flask and make to volume with the pH 8 buffer. Assay these solutions as directed in paragraph (b)(1) of this section.

(vi) *Calculations.*

$$\text{Total gentamicins} = \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} + \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} + \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977}$$

$$\text{Percent of gentamicin } C_1 = \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} \times \frac{100}{\text{Total gentamicins}}$$

$$\text{Percent of gentamicin } C_2 = \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} \times \frac{100}{\text{Total gentamicins}}$$

$$\text{Percent of gentamicin } C_{1a} = \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977} \times \frac{100}{\text{Total gentamicins}}$$

where:

The assays are expressed in terms of the microgram equivalents of gentamicin; and

The factors 0.786, 1.023, and 0.977 represent the activities of gentamicins C_1, C_2, and C_{1a} relative to the gentamicin activity of the gentamicin master standard.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(2) of that section.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.30 Kanamycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate is the sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on an anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 4 percent.

(iv) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives a positive identity test for kanamycin.

(vii) It contains not more than 5.0 percent kanamycin B.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, identity, kanamycin B content, and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water, and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol; then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(7) *Kanamycin B content*—(i) *Cylinders* (*cups*). Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make agar for the base and seed layers as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
pH 7.8 to 8.0 after sterilization.	
Distilled water, q.s	1,000.00 ml.

(iii) *Working standard.* Dissolve a suitable quantity of the kanamycin sulfate working standard, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration equivalent to 1.0 milligram of kanamycin per milliliter.

(iv) *Preparation of sample.* To 100 milligrams, accurately weighed, of kanamycin sulfate in a suitable container (such as a 7.5-milliliter serum vial) add 5.0 milliliters of 6*N* hydrochloric acid, and tightly close the container. Heat in a water bath at 100° C. for 1 hour and cool. Add 4 milliliters of 6*N* sodium hydroxide, then dilute with sterile 0.1*M* potassium phosphate buffer, pH 8.0, to obtain a concentration of the equivalent of 1 microgram of kanamycin per milliliter (estimated).

(v) *Preparation of test organism.* Use *Bacillus subtilis* (ATCC 6633)[1] prepared as described in § 436.103 of this chapter, using method 2.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in paragraph (b)(7) of this section to each Petri dish (20 millimeters × 100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the fresh daily inoculum described in paragraph (b)(7)(iv) of this section to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare on the day of testing in 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, from the standard stock solution, sufficient volumes of the following concentrations: 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter. The 1.0 microgram-per-milliliter solution is the reference point of the standard curve. On each of three plates fill three cylinders with the 1.0 microgram-per-milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1.0-microgram determinations for each of the other points on the curve. After the plates have incubated read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram-per-milliliter concentration and the readings of the concentration test for each set of three plates and average also all 36 readings of the 1.0 microgram-per-milliliter concentration. The average of the 36 readings of the 1.0 microgram-per-milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram-per-milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram-per-milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram-per-milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram-per-milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram-per-milliliter concentration, on 2-cycle semilogarithmic paper, using the concentration in micrograms per milliliter as the ordinate and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

where:

L=Calculated zone diameter for the lowest concentration of the standard curve;

H=Calculated zone diameter for the highest concentration of the standard curve;

c=Average zone diameter of 36 readings of the 1.0 microgram-per-milliliter standard;

a, b, d, e=Corrected average values for the 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms-per-milliliter solutions, respectively.

Plot the values obtained for *L* and *H* and connect with a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in paragraph (b)(7)(vi) of this section, so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram-per-milliliter standard and three cylinders with the 1.0 microgram (estimated)-per-milliliter sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of kanamycin B content.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the average of the standard, add the difference between them to the

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

1.0-microgram zone size of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0-microgram value on the curve. From the curve, read the kanamycin potencies corresponding to these corrected values of zone sizes. Multiply the observed potency by 100 and divide by 126 to obtain a value representing the potency in terms of the milligram equivalent of kanamycin B. The calculated amount of kanamycin B is not more than 5 percent of the content of kanamycin found in paragraph (b)(1) of this section.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.30a Sterile kanamycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Kanamycin sulfate is the sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on an anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 4 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not more than 1.0 percent.

(viii) It gives a positive identity test for kanamycin.

(ix) It contains not more than 5.0 percent kanamycin B.

(x) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, identity, crystallinity, and kanamycin B content.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of kanamycin per milliliter.

(5) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(7) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(8) *Identity*. Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol. Then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(9) *Kanamycin B content*. Proceed as directed in § 444.30(b)(7).

(10) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.42 Neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 600 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) It gives a positive identity test for neomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 33 milligrams of neomycin per milliliter.

(5) *Identity*—(i) *Reagents.* (*a*) Sulfuric acid solution: Mix concentrated sulfuric acid and distilled water in volumetric proportions of 40:60.

(*b*) Xylene.

(*c*) *p*-Bromoaniline: (Prepare and store this reagent in brown, nonactinic glassware.) Place 380 milliliters of thiourea-saturated glacial acetic acid solution in the bottle, add 10 milliliters of 20 percent sodium chloride solution, 5 milliliters of 5 percent oxalic acid solution, and 5 milliliters of 10 percent disodium phosphate solution, and mix well. Add 8 grams of *p*-bromoaniline and mix well. Let this reagent stand overnight before use. Prepare the reagent once weekly.

(ii) *Procedure.* Place about 10 milligrams of the sample into a test tube (19 millimeters × 150 millimeters), dissolve with 1 milliliter of water, and then carefully add 5 milliliters of the sulfuric acid solution. Heat in a boiling water bath for 100 minutes. Cool to room temperature. Add 10 milliliters of xylene to the test tube. Stopper the tube and shake vigorously for about 1 minute. Let the two layers separate and then decant the xylene layer into a second test tube. Add 10 milliliters of the *p*-bromoaniline reagent to the xylene solution, shake, and let stand. The development of a vivid pink-red color is a positive identity test for neomycin.

[40 FR 22252, May 22, 1975, as amended at 50 FR 19919, May 13, 1985]

§ 444.42a Sterile neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 8.0 percent.

(vi) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) It gives a positive identity test for neomycin.

(2) *Labeling.* It is to be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required;

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods:

(i) *Plate assay using Staphylococcus epidemmidis* (*ATCC 12228*)[1]—(*a*) *Cylinders* (*cups*). Use cylinders described in § 440.80a(b)(1)(i) of this chapter.

(*b*) *Culture media*. Using ingredients that conform to the standards prescribed by the U.S.P. or N.F.:

(*1*) Make nutrient agar for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water q.s	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*2*) Make nutrient agar for the base and seed layers as described in paragraph (b)(1)(i)(*b*)(*1*) of this section, except that its pH after sterilization is 7.8 to 8.0.

In lieu of preparing the media from the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section, they may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in paragraph (b)(1)(i)(*b*) of this section are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard*. Dry a portion of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. When stored under refrigeration, the stock solution may be used for a period not exceeding 2 weeks.

(*d*) *Preparation of sample*. Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with sufficient solution 3 to obtain a reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(*e*) *Preparation of test organism*. The test organism is *Staphylococcus epidermidis* (ATCC 12228),[1] which is maintained on slants of nutrient agar described in (b)(1)(i)(*b*)(*1*) of this section. Using 3 milliliters of U.S.P. saline T.S., wash the organism from the nutrient agar slant (which has been incubated for 24 hours at 32° C.−35° C.) onto a large nutrient agar surface such as that provided by a Roux bottle containing 300 milliliters of nutrient agar. Incubate for 24 hours at 32° C.−35° C. Wash the resulting growth from the nutrient surface, using 50 milliliters of sterile U.S.P. saline T.S. Adjust the volume of the suspension so that a 1:14 dilution will give 25 percent light transmission when measured with a suitable photo-electric colorimeter having a 580 mμ filter and a 13-millimeter diameter test tube as an absorption cell. By the use of test plates, determine the appropriate inoculum of the adjusted suspension (usually 0.1 milliliter) to be inoculated to each 100 milliliters of seed layer agar in order to obtain satisfactory zones of inhibition. The suspension may be used for 1 week if stored under refrigeration.

(*f*) *Preparation of plates*. Add 21 milliliters of the agar prepared as described in paragraph (b)(1)(i)(*b*)(*2*) of this section to each Petri dish (20 millimeters × 100 millimeters). Distribute the agar evenly in the plates and allow to harden on a level surface. Accurately measure a sufficient quantity of the nutrient agar, cool to 48° C., and add the appropriate inoculum of the adjusted suspension, prepared as described in paragraph (b)(1)(i)(*e*) of this section. Swirl the inoculated nutrient agar to obtain a homogeneous suspension, and add 4 milliliters to each of the plates containing the 21 milliliters of uninoculated nutrient agar. Tilt the plates back and forth to spread the inoculated nutrient agar evenly, and allow to harden on a level surface. After the agar has hardened, place six cylinders described in paragraph

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(b)(1)(i)(*a*) of this section on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use the plates the same day they are prepared.

(*g*) *Standard curve.* Using the stock solution of the working standard prepared as described in paragraph (b)(1)(i)(*c*) of this section, prepare solutions in 0.1*M* potassium phosphate buffer pH 8.0 of the following concentrations: 0.64, 0.8, 1.0, 1.25, 1.56 micrograms of neomycin per milliliter. The 1.0 microgram per milliliter concentration is the reference concentration of the assay. Use a total of 12 plates, three plates for each solution except the reference point solution which is included on each plate. On each of the three plates, fill three cylinders with the reference point solution and the other three cylinders with the concentrations under test. Thus, there will be 36 reference point determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the reference point concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the reference point concentration. The average of the 36 readings of the reference point concentration is the correction point of the curve. Correct the average value obtained for each point to the figure it would be if the reference point reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter (reference point) concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of the set of three plates (the 0.8 microgram per milliliter set) is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points either by inspection or by means of the following equations:

$$H=(3a+2b+c-e)/(5),$$
$$L=(3e+2d+c-a)/(5),$$

where:

L=Calculated zone diameter for the lowest concentration of the standard curve;

H=Calculated zone diameter for the highest concentration of the standard curve;

c=Average zone diameter of 36 readings of the reference point standard solution;

a, b, d, e=Corrected average values for the other standard solutions, lowest to highest concentrations, respectively.

(*h*) *Assay procedure.* Use three plates for each sample. Fill three cylinders on each plate with the standard and three cylinders with the sample, which has been diluted to the reference concentration, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 32° C.–35° C., and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the reference point zone of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the reference point value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Plate assay using Staphylococcus aureus (ATCC 6538P).*[1] Proceed as directed in paragraph (b)(1)(i) of this section except that the reference concentration of the sample under test is 10.0 micrograms of neomycin per milliliter; the concentrations of the standard curve solutions are 6.4, 8.0, 10.0, 12.5, 15.6 micrograms of neomycin per milliliter; and the suspension of the test organism, staphylococcus aureus (ATCC 6538P),[1] is adjusted so that a 1:19

[1] Available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852.

dilution will give 25 percent light transmission and the usual inoculum for each 100 milliliters of agar for the seed layer is 0.2 milliliter of diluted suspension.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 440.80a(b)(3) of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution containing 10 milligrams of neomycin per milliliter in pyrogen-free, sterile U.S.P. saline T.S.

(4) [Reserved]

(5) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorus pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(6) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 33 milligrams of neomycin per milliliter.

(7) *Identity*—(i) *Reagents.* (*a*) Sulfuric acid solution: Mix concentrated sulfuric acid and distilled water in volumetric proportions of 40:60.

(*b*) Xylene.

(*c*) *p*-Bromoaniline: (Prepare and store this reagent in brown, nonactinic glassware.) Place 380 milliliters of thioureasaturated glacial acetic acid solution in the bottle, add 10 milliliters of 20 percent sodium chloride solution, 5 milliliters of 5 percent oxalic acid solution, and 5 milliliters of 10 percent disodium phosphate solution, and mix well. Add 8 grams of *p*-bromoaniline and mix well. Let this reagent stand overnight before use. Prepare the reagent once weekly.

(ii) *Procedure.* Place about 10 milligrams of the sample into a test tube (19 millimeters × 150 millimeters), dissolve with 1 milliliter of water, and then carefully add 5 milliliters of the sulfuric acid solution. Heat in a boiling water bath for 100 minutes. Cool to room temperature. Add 10 milliliters of xylene to the test tube. Stopper the tube and shake vigorously for about 1 minute. Let the two layers separate and then decant the xylene layer into a second test tube. Add 10 milliliters of the *p*-bromoaniline reagent to the xylene solution, shake, and let stand. The development of a vivid pink-red color is a positive identity test for neomycin.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985; 53 FR 12660, Apr. 15, 1988]

§ 444.46 Netilmicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Netilmicin sulfate is the sulfate salt of D-Streptamine,4-*O*-[3-amino-6-(aminomethyl)-3,4-dihydro-2*H*-pyran-2-yl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-N^1-ethyl-, (2*S*-*cis*)-, (2:5). It is a white-to-buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 595 micrograms of netilmicin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 15.0 percent.

(iii) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(iv) Its residue on ignition is not more than 1.0 percent.

(v) Its specific rotation in an aqueous solution containing 30 milligrams per milliliter at 25° C is not less than +88° and not more than +96°.

(vi) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH,

residue on ignition, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of netilmicin per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(c) of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams per milliliter.

(4) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(5) *Specific rotation*. Use an aqueous solution containing 3 milligrams of sample per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0–decimeter tube, and calculate the specific rotation on an anhydrous basis.

(6) *Identity*. Proceed as directed in § 436.318 of this chapter, except:

(i) Prepare sample and standard solutions containing 10 milligrams of netilmicin per milliliter;

(ii) Use 5 microliters of the solutions to spot the chromatography plate;

(iii) Remove the plate from the tank after 1.5 hours; and

(iv) Netilmicin sulfate appears as a brown spot.

[48 FR 18800, Apr. 26, 1983; 48 FR 22144, May 17, 1983, as amended at 55 FR 11584, Mar. 29, 1990]

§ 444.50 Paromomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Paromomycin sulfate is the sulfate salt of a kind of paromomycin or a mixture of two or more such salts. It is a creamy-white to light-yellow powder. It is so purified and dried that:

(i) Its potency is not less than 675 micrograms per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) The pH of a 3.0 percent aqueous solution is not less than 5.0 and not more than 7.5.

(v) Its specific rotation at 25° C. in water is not less than +50° and not more than +55° on an anhydrous basis.

(vi) Its residue on ignition is not more than 2.0 percent.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, specific rotation, and residue on ignition.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a 3.0 percent aqueous solution.

(5) *Specific rotation*. Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter volumetric flask. Dissolve in a few milliliters of water, add water to volume, and mix. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(6) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§444.62 Sisomicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sisomicin sulfate is the sulfate salt of *O*-3-deoxy-4-*C*-methyl-3-(methylamino)–β–L–arabinopyranosyl(1→4)-*O*-[2,6-diamino-2,3,4,6-tetradeoxy-α-D-*glycero*- hex-4-enopyranosyl(1→6)-2-deoxy-L-streptamine. It is a hygroscopic powder. It is so purified and dried that:

(i) Its potency is not less than 580 micrograms of sisomicin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 15.0 percent.

(iv) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25° C is not less than +100° and not more than +110°.

(vii) It gives a positive identity test for sisomicin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, specific rotation, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay.* Sisomicin is hygroscopic and care should be exercised during storage and weighing of samples.

(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of sisomicin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(c) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 40 milligrams of sisomicin per milliliter.

(5) *Residue on ignition.* Proceed as directed in §436.207(a) of this chapter.

(6) *Specific rotation.* Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in §436.210 of this chapter, using a 1.0 decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(7) *Identity.* Proceed as directed in §436.318 of this chapter, except:

(i) Prepare sample and standard solutions containing 10 milligrams of sisomicin per milliliter;

(ii) Use 5 microliters of the solutions to spot the chromatographic plates;

(iii) Remove the plate from the tank after 3 hours; and

(iv) The compound appears as a brown spot.

[46 FR 2988, Jan. 13, 1981; 46 FR 16676, Mar. 13, 1981; 46 FR 22359, Apr. 20, 1981, as amended at 50 FR 19919, May 13, 1985]

§444.70a Sterile streptomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile streptomycin sulfate is the sulfate salt of a kind of streptomycin or a mixture or two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 650 micrograms and not more than 850 micrograms of streptomycin per milligram. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of streptomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 200 milligrams per milliliter

is not less than 4.5 and not more than 7.0.

(viii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in manufacturing another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration; and also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic syringe and needle, remove all of the withdrawable contents from each container represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representative portion from each container. Accurately dilute the sample thus obtained with sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30 micrograms of streptomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of streptomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 200 milligrams per milliliter.

(8) *Identity*—(i) *Reagents. (a)* 10 percent ferric chloride stock solution: Dissolve 5 grams of $FeCl_3 \bullet 6H_2O$ in 50 milliliters of 0.1*N* HCl.

(*b*) 0.25 percent ferric chloride solution: Dilute 2.5 milliliters of 10 percent ferric chloride in 0.1*N* HCl to 100 milliliters with 0.01*N* HCl. Prepare the solution fresh daily.

(ii) *Procedure.* Using distilled water, dilute the sample to be tested to a concentration of approximately 1,000 micrograms per milliliter. To 5.0 milliliters of this solution, add 2.0 milliliters of 1*N* NaOH and heat in a boiling water bath for 10 minutes. Cool in the ice water for 3 minutes and then acidify the solution by adding 2.0 milliliters of 1.2N HCl. Add 5.0 milliliters of 0.25 percent ferric chloride reagent. A violet color indicates the presence of streptomycin.

[42 FR 21275, Apr. 26, 1977, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19919, May 13, 1985]

§ 444.80 Tobramycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin is *0*-3-amino-3-deoxy-α-*D*-glucopyranosyl-(1→4)-*0*-[2,6-diamino-2,3,6-trideoxy-α-*D-ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of tobramycin per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 9 and not more than 11.

(v) It gives a positive identity test for tobramycin.

(vi) Its residue on ignition is not more than 1.0 percent.

(vii) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, residue on ignition, and heavy metals.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive:

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(ii) *Nonaqueous titration.* Proceed as directed in § 436.213 of this chapter, using the titration procedure described in paragraph (e)(2) of that section. Calculate the tobramycin content as follows:

Micrograms tobramycin per milligram = $[(A-B)\times(\text{normality of perchloric acid reagent})\times 93.4\times 100\times 1{,}000]/(\text{Weight of sample in milligrams}\times(100-m))$

where:

A=Milliliters of perchloric acid reagent used in titrating the sample;

B=Milliliters of perchloric acid reagent used in titrating the blank;

m=Percent moisture of the sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.318 of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[40 FR 57798, Dec. 12, 1975, as amended at 45 FR 16476, Mar. 14, 1980; 50 FR 19919, May 13, 1985]

§ 444.81a Sterile tobramycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile tobramycin sulfate is the sulfate salt of *0*-3-amino-3-deoxy-α-*D*-glucopyranosyl-(1→4)-*0*-[2,6-diamino-2,3,6-trideoxy-α-*D-ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine. It is a lyophilized powder. It is so purified and dried that:

(i) Its potency is not less than 634 micrograms and not more than 739 micrograms of tobramycin per milligram on an ''as is'' basis. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of tobramycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 2.0 percent.

(vi) Its pH in an aqueous solution containing 40 milligrams per milliliter, or when reconstituted as directed in the labeling, is not less than 6.0 and not more than 8.0.

(vii) It gives a positive identity test for tobramycin.

(viii) Its residue on ignition is not more than 1.0 percent.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, residue on ignition, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufcture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 14 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample with sufficient sterile distilled water to obtain a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sterile distilled water to obtain a stock solution of convenient concentration. Further dilute a portion of the stock solution with sterile distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of tobramycin per milliliter.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams per milliliter, or if it is packaged for dispensing, reconstitute as directed in the labeling.

(7) *Identity*. Proceed as directed in § 436.318 of this chapter.

(8) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(9) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

[44 FR 26072, May 4, 1979, as amended at 45 FR 16476, Mar. 14, 1980; 50 FR 19919, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 444.130 Kanamycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Kanamycin sulfate capsules are composed of crystalline kanamycin sulfate, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in gelatin capsules. Each capsule contains 500 milligrams of kanamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of kanamycin that it is represented to contain. The loss on drying is not more than 4.0 percent. The crystalline kanamycin sulfate used conforms to the standards prescribed by § 444.30(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, identity, kanamycin B content, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) Kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed

glass blender jar with sufficient sterile distilled water to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.142 Neomycin sulfate oral dosage forms.

§ 444.142a Neomycin sulfate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate tablets are tablets composed of neomycin sulfate with one or more suitable and harmless binders, and with or without one or more suitable and harmless fillers, buffers, lubricants, and colorings. Each tablet contains 150 milligrams, 175 milligrams, or 350 milligrams of neomycin. The moisture content is not more than 10.0 percent. Tablets shall disintegrate within 1 hour. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample as follows: Place a representative number of tablets into a high-speed glass blender, add a sufficient quantity of 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its neomycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 440.180a(b)(3) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 46 FR 25608, May 8, 1981; 50 FR 19919, May 13, 1985]

§ 444.142b Neomycin sulfate oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate oral solution is neomycin sulfate with or without one or more suitable and harmless flavorings, colorings, and preservatives in an aqueous vehicle. Each milliliter contains 17.5 milligrams of neomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain. Its pH is not less than 5.0 and not more than 7.5. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, except prepare the sample as follows: Remove an accurately measured representative portion with a suitable syringe, and dilute with sufficient 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.150 Paromomycin sulfate oral dosage forms.

§ 444.150a Paromomycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Paromomycin sulfate capsules are paromomycin sulfate enclosed in a suitable and harmless gelatin capsule. Each capsule contains 250 milligrams of paromomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of paromomycin that it is represented to contain. The loss on drying is not more than 7.0 percent. The paromomycin sulfate used conforms to the standards prescribed therefor by § 444.50(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The paromomycin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, and residue on ignition.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The paromomycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules for 3 to 5 minutes in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.150b Paromomycin sulfate sirup.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Paromomycin sulfate sirup contains the equivalent of 25 milligrams of paromomycin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of paromomycin that it is represented to contain. It may contain one or more suitable and harmless solvents, flavorings, colorings, preservatives, and buffers in water. Its pH is not less than 7.5 and not more than 8.5. The paromomycin sulfate used conforms to the requirements of § 444.50(a)(1) (i), (ii), (iv), (v), and (vi).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays for:

(*a*) The paromomycin sulfate used in making the batch for potency, pH, specific rotation, and residue on ignition.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The paromomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove an appropriate aliquot of the sirup and transfer to an appropriate-sized volumetric flask. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and mix well. Further dilute with solution 3 to the reference concentration of 1.0 microgram of paromomycin per milliliter (estimated).

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 444.206 Amikacin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Amikacin sulfate injection is an aqueous solution of amikacin with suitable and harmless buffer substances and preservatives. Each milliliter contains amikacin sulfate equivalent to either 50 milligrams or 250 milligrams of amikacin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amikacin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 3.5 and not more than 5.5. The amikacin used conforms to the standards prescribed by § 444.6(a)(1) or, if amikacin sulfate is used, to the standards prescribed by § 444.7(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amikacin used in making the batch for potency, moisture, pH, identity, residue on ignition, specific rotation, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The amikacin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask and dilute to volume with sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10.0 micrograms of amikacin per milliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 25 milligrams of amikacin per milliliter.

(4) [Reserved]

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[41 FR 49483, Nov. 9, 1976, as amended at 50 FR 19919, May 13, 1985; 55 FR 38677, Sept. 20, 1990]

§ 444.220 Gentamicin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Gentamicin sulfate injection is an aqueous solution of gentamicin sulfate with or without one or more suitable buffers, sequestering agents, tonicity agents, or preservatives. Each milliliter contains gentamicin sulfate equivalent to either 0.4, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.6, 2.0, 2.4, 10.0, or 40 milligrams of gentamicin. Its potency is satisfactory if it contains

not less than 90 percent nor more than 125 percent of the number of milligrams of gentamicin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 3.0 nor more than 5.5. The gentamicin sulfate used conforms to the standards prescribed by § 444.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1,C_{1a},C_2, and identity.

(*b*) The batch for gentamicin potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 40 containers if each milliliter contains the equivalent of 2.0 milligrams or 10.0 milligrams of gentamicin; a minimum of 12 containers if each milliliter contains the equivalent of 40.0 milligrams of gentamicin; or, a minimum of 10 containers if each milliliter contains the equivalent of 1.0 milligram of gentamicin.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), dilute an accurately measured representative portion of the product to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 10 milligrams of gentamicin per kilogram, but not to exceed 10 milliliters per kilogram.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[39 FR 19046, May 30, 1974, as amended at 46 FR 2994, Jan. 13, 1981; 46 FR 16683, Mar. 13, 1981; 46 FR 31009, June 12, 1981; 47 FR 56490, Dec. 17, 1982; 48 FR 44775, Sept. 30, 1983; 49 FR 49287, Dec. 19, 1984; 50 FR 10754, Mar. 18, 1985; 50 FR 19919, May 13, 1985]

§ 444.230 Kanamycin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate injection is an aqueous solution of kanamycin sulfate with suitable and harmless buffer substances and preservatives. It contains either 75 milligrams of kanamycin per 2.0 milliliters, or 250 milligrams of kanamycin per milliliter, or 1.0 gram of kanamycin per 3.0 milliliters. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of kanamycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 3.5 and not more than 5.0. The kanamycin sulfate used conforms to the standards prescribed by § 444.30a(a)(1)(i), (v), (vii), (viii), (ix), and (x).

(2) *Labeling.* In addition to the requirements prescribed by § 432.5 of this chapter, the labeling of each package shall bear a warning to the effect that older patients and patients receiving a total dose of more than 20 grams of the drug should be carefully observed for signs of eighth-nerve damage. In patients with impaired kidney function or with prerenal azotemia, the risk of severe ototoxic reaction that may result in permanent deafness is sharply increased.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, residue on ignition, loss on drying, identity, crystallinity, and kanamycin B content.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative aliquot of the sample into an appropriate-sized volumetric flask and dilute to volume with sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of kanamycin per milliliter.

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.246 Netilmicin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Netilmicin sulfate injection is an aqueous solution of netilmicin sulfate and one or more buffers, chelating agents, antioxidants, and preservatives. Each milliliter contains netilmicin sulfate equivalent to 10 milligrams, 25 milligrams, or 100 milligrams of netilmicin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of netilmicin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 3.5 and not more than 6.0. The netilmicin sulfate used conforms to the standards prescribed by § 444.46(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The netilmicin sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, and identity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The netilmicin sulfate used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the product with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of netilmicin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of netilmicin per milliliter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[48 FR 18801, Apr. 26, 1983, as amended at 55 FR 11584, Mar. 29, 1990]

§ 444.262 Sisomicin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sisomicin sulfate injection is an aqueous solution of sisomicin sulfate and one or more suitable buffers, chelating agents, and preservatives.

Each milliliter contains sisomicin sulfate equivalent to 50 milligrams of sisomicin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of sisomicin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 2.5 and not more than 5.5. The sisomicin sulfate used conforms to the standards prescribed by § 444.62(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sisomicin sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, and identity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The sisomicin sulfate used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 vials.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the product with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of sisomicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of sisomicin per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[46 FR 2989, Jan. 13, 1981, as amended at 50 FR 19919, May 13, 1985]

§ 444.270 Streptomycin sulfate injectable dosage forms.

§ 444.270a Sterile streptomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile streptomycin sulfate, packaged for dispensing, are described in § 444.70a.

[42 FR 21275, Apr. 26, 1977]

§ 444.270b Streptomycin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate injection is an aqueous solution of streptomycin sulfate. It may contain one or more suitable and harmless preservatives, buffer substances and stabilizing agents. Each milliliter contains streptomycin sulfate equivalent to 400 milligrams, 420 milligrams, or 500 milligrams of streptomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of streptomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its pH is not less than 5.0 and not more than 8.0. The streptomycin sulfate used conforms to the standards prescribed by § 444.70a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain;

(i) Results of tests and assays on:

(*a*) The streptomycin sulfate used in making the batch for potency, depressor substances, loss on drying, pH, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances (except that the results of this test performed on the streptomycin sulfate used in making the batch may be submitted instead), and pH.

(ii) Samples required:

(*a*) The streptomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) If the batch is packaged for use in the manufacture of another drug:

(*i*) For all tests except sterility: Five containers, each containing not less than 2.0 milliliters.

(*ii*) For sterility testing: 20 containers, each containing not less than 2.0 milliliters.

(*2*) If the batch is packaged for dispensing:

(*i*) For all tests except sterility: A minimum of eight immediate containers.

(*ii*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and method of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic syringe and needle, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Accurately dilute the portion with sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30 micrograms of streptomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of streptomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances* (the depressor substances test may be omitted if it is performed on the streptomycin sulfate used in preparing the injection). Proceed as directed in § 436.35 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[42 FR 21275, Apr. 26, 1977; 42 FR 37543, July 7, 1977, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19919, May 13, 1985]

§ 444.280 Tobramycin sulfate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tobramycin sulfate injection is tobramycin solubilized with sulfuric acid in an aqueous solution containing one or more suitable buffers, chelating agents, and preservatives. Each milliliter contains tobramycin sulfate equivalent to either 10 milligrams or 40 milligrams of tobramycin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 3.0 and not more than 6.5. The tobramycin used conforms to the standards prescribed by § 444.80(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tobramycin used in making the batch for potency, moisture, pH, identity, residue on ignition, and heavy metals.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 40 vials if each milliliter contains 10 milligrams of tobramycin per milliliter, or a minimum of 12 vials if each milliliter contains the equivalent of 40 milligrams of tobramycin.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: If the immediate container is a single-dose vial, use a suitable hypodermic needle and syringe and remove all the withdrawable contents; or, if the labeling specifies the amount of potency in a given volume,

remove an accurately measured representative portion from each container. Dilute this portion with sufficient distilled water to give a stock solution of convenient concentration. If the preparation is packaged in a prefilled syringe, eject the entire contents of the syringe and dilute with distilled water to obtain a stock solution of convenient concentration. Further dilute the stock solution to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of tobramycin per milliliter.

(4) [Reserved]

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[40 FR 57798, Dec. 12, 1975, as amended at 50 FR 19919, May 13, 1985]

§ 444.281 Sterile tobramycin sulfate.

The requirements for certification and the tests and methods of assay for sterile tobramycin sulfate packaged for dispensing are described in § 444.81a.

[44 FR 26072, May 4, 1979]

Subpart D—Ophthalmic Dosage Forms

§ 444.320 Gentamicin sulfate ophthalmic dosage forms.

§ 444.320a Gentamicin sulfate ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ophthalmic solution contains in each milliliter the equivalent of 3.0 milligrams of gentamicin and suitable buffers and preservatives. Its potency is satisfactory if it is not less than 90 and not more than 135 percent of the number of milligrams of gentamicin it is represented to contain. It is sterile. Its pH is not less than 6.5 nor more than 7.5. The gentamicin sulfate conforms to the standards prescribed by § 444.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the product with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.320b Gentamicin sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ointment contains in each gram the equivalent of 3.0 milligrams of gentamicin with suitable preservatives in a white petrolatum base. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin

that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for particulate contamination. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency, sterility, moisture, and particulate contamination.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, except prepare the sample as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of solution 3. Repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Particulate contamination.* Proceed as directed in § 436.206 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.320c Gentamicin sulfate-prednisolone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate-prednisolone acetate ophthalmic suspension is an aqueous suspension containing in each milliliter gentamicin sulfate equivalent to 3.0 milligrams of gentamicin and 10.0 milligrams of prednisolone acetate. It contains suitable and harmless chelating agents, tonicity agents, buffers, and preservatives. Its gentamicin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of gentamicin that it is represented to contain. Its prednisolone acetate content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of prednisolone acetate that it is represented to contain. Its pH is not less than 5.4 and not more than 6.6. It is sterile. The gentamicin sulfate used conforms to the standards prescribed by § 444.20(a)(1). The prednisolone acetate used conforms to the standards prescribed by the USP XXI.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicin C_1, C_{1a}, C_2, and identify.

(B) The prednisolone acetate used in making the batch for all USP XXI specifications.

(C) The batch for gentamicin content, prednisolone acetate content, sterility, and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Gentamincin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Prednisolone acetate content.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with octadecyl hydrocarbon bonded silicas, a flow rate of 2.0 milliliters per minute, and an injection volume of 30 microliters. Mobile phase, reference standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Mix acetonitrile distilled deionized water (40:60). Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter.

(ii) *Reference standard and sample solutions*—(A) *Preparation of reference standard solution.* Accurately weigh approximately 60 milligrams of prednisolone acetate reference standard into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol and mix well. Transfer 8 milliliters of this solution into a 50-milliliter volumetric flask, dilute to volume with 70 percent methyl alchohol, and mix well.

(B) *Preparation of sample solution.* Transfer 1.0 milliliter of the sample into a 50-milliliter volumetric flask, dilute to volume with 70 percent methyl alcohol, and mix well.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 1.25 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 2,000 theoretical plates.

(C) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the milligrams of prednisolone acetate per milliliter of sample as follows:

$$\text{Milligrams of prednisolone acetate} = \frac{A_u \times C_s \times d}{A_s}$$

where:

A_u=Area of the prednisolone acetate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the prednisolone acetate peak in the chromatogram of the prednisolone acetate reference standard;

C_s=Concentration of prednisolone acetate in the reference standard solution in milligrams per milliliter; and

d=Dilution factor of the sample.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[53 FR 40725, Oct. 18, 1988, as amended at 59 FR 8398, Feb. 22, 1994]

§ 444.320d Gentamicin sulfate-prednisolone acetate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate-prednisolone acetate ophthalmic ointment contains in each gram gentamicin sulfate equivalent to 3.0 milligrams of gentamicin and 6.0 milligrams of prednisolone acetate, with a suitable lubricant and preservative in a suitable and harmless white petrolatum base. Its gentamicin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of gentamicin that it is represented to contain. Its prednisolone acetate content is satisfactory if it is

not less than 90 percent and not more than 110 percent of the number of milligrams of prednisolone acetate that it is represented to contain. It is sterile. Its moisture content is not more than 2.0 percent. It passes the test for metal particles. The gentamicin sulfate used conforms to the standards prescribed by § 444.20(a)(1). The prednisolone acetate used conforms to the standards prescribed by the United States Pharmacopeia.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, C_2, and identity.

(B) The prednisolone acetate used in making the batch for all USP XXI specifications.

(C) The batch for gentamicin content, prednisolone acetate content, sterility, moisture, and metal particles.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) *The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.*

(B) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Gentamicin content.* Proceed as directed in § 436.105 of this chapter, except prepare the sample as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of solution 3. Repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Prednisolone acetate content.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with octadecyl hydrocarbon bonded silicas 3 to 10 micrometers in diameter, a flow rate of 2.0 milliliters per minute, and an injection volume of 30 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Mobile phase.* Mix acetonitrile distilled deionized water (40:60). Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(B) *Internal standard solution.* Accurately weigh 135 milligrams ± 10 milligrams of fluorometholone acetate into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol.

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Prepare the working standard solution fresh before injection by dissolving approximately 40 miligrams ± 2 milligrams of prednisolone acetate, accurately weighed, into a 100-milliliter volumetric flask with 25 milliliters of methyl alcohol. Sonicate to dissolve and dilute to volume with methyl alcohol and mix well. Transfer 8 milliliters of this solution into a 50-milliliter volumetric flask. Add 25 milliliters of hexane and shake. Add 2.0 milliliters of internal standard as described in paragraph (b)(2)(i)(B) of this section, and dilute to volume with methyl alcohol. Shake vigorously for 30 seconds, allow the phases to separate, then aspirate the upper hexane layer and dilute to volume with methyl alcohol. Centrifuge for 10 minutes at 5,700 revolutions per minute.

(B) *Sample solution.* Accurately weigh 500 milligrams ± 20 milligrams of the sample into a 50-milliliter volumetric

flask. Add 25 milliliters of hexane and sonicate. Add 2.0 milliliters of the internal standard. Dilute to volume with methyl alcohol. Shake vigorously for 30 seconds and allow the phase to separate. Aspirate the upper hexane and cloudy layers. Dilute to volume with methyl alcohol. Centrifuge for 10 minutes at 5,700 revolutions per minute.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 1.50 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 2,500 theoretical plates.

(C) *Resolution.* The resolution (R) between the peak for prednisolone acetate and the internal standard is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided comparable system suitability requirements are met. However, the sample preparation described in paragraph (b)(2)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the percent of prednisolone acetate as follows:

$$\text{Percent of prednisolone acetate (w/w)} = \frac{R_u \times P_s \times d \times 100}{R_s \times W_u}$$

where:

R_u=Area of the prednisolone acetate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the prednisolone acetate peak in the chromatogram of the prednisolone acetate working standard /Area of internal standard peak;

P_s=Prednisolone acetate activity in the prednisolone acetate working standard solution in milligrams per milliliter;

W_u=Weight of sample in milligrams; and≤

d=Dilution factor of the sample.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(3).

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[55 FR 2643, Jan. 26, 1990]

§ 444.342 Neomycin sulfate ophthalmic dosage forms.

§ 444.342a Neomycin sulfate- ———— ophthalmic suspension; neomycin sulfate- ———— ophthalmic solution (the blanks being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension or a solution containing, in each milliliter, 3.5 milligrams of neomycin and the following other active ingredients in a suitable and harmless vehicle:

(i) 15 milligrams of cortisone acetate; or

(ii) 5 milligrams or 25 milligrams of hydrocortisone acetate; or

(iii) 1 milligram or 2 milligrams of prednisolone; or

(iv) 1 milligram of sodium dexamethasone phosphate; or

(v) 5 milligrams of prednisolone phosphate.

It contains suitable and harmless buffers, dispersants, and preservatives. It is sterile. Its pH is not less than 6.0 and not more than 8.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 containers, each

containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §444.442a(b)(1). Its neomycin content is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH*. Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 47 FR 23441, May 28, 1982; 50 FR 19919, May 13, 1985; 59 FR 8398, Feb. 22, 1994]

§444.342b Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution is a solution containing in each milliliter, 1.75 milligrams of neomycin, 10,000 units of polymyxin B and 0.025 milligram of gramicidin, and with one or more suitable and harmless buffers, dispersants, and preservatives in a suitable and harmless isotonic aqueous vehicle. It is sterile. Its pH is not less than 4.7 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1)(i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1)(i), (vi), (vii), and (ix) of this chapter. The gramicidin used conforms to the standards prescribed by §448.25(a)(1)(i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples*. In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The gramicidin used in making the batch for potency, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for neomycin content, polymyxin content, gramicidin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*d*) The batch:

(*1*) For all tests except sterility: A minimum of 7 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, except that if the product is sterilized after filling, a representative sample consisting of 10 immediate containers from each sterilizer load. If only 1 sterilizer load is involved, the sample shall consist of 20 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.42a(b)(1), except prepare the sample as follows: Remove an accurately measured portion and dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Remove an accurately measured portion and dilute with 10 percent potassium phosphate buffer, pH 6.0, to a reference concentration of 10 units of polymyxin per milliliter. Proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter. The polymyxin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except to prepare the sample for assay remove a representative sample with a suitable syringe, place into an appropriate volumetric flask, and dilute with alcohol U.S.P. XX to obtain a stock solution of convenient concentration. Make proper estimated dilutions in alcohol U.S.P. XX to the reference concentration. The gramicidin content is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 47 FR 22515, May 25, 1982; 47 FR 23441, May 28, 1982; 47 FR 23709, June 1, 1982; 50 FR 19919, May 13, 1985]

§ 444.342c Neomycin sulfate-gramicidin ———— ophthalmic solution; neomycin sulfate-gramicidin ———— ophthalmic suspension (the blanks being filled in with the established name(s) of the other ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a solution or suspension in a suitable and harmless aqueous vehicle containing, in each milliliter, the following:

(i) 2.5 milligrams of neomycin, 0.025 milligram of gramicidin, and 1 milligram of fluorocortisone acetate; or

(ii) 2.5 milligrams of neomycin, 0.025 milligram of gramicidin, and 1.14 milligrams of fluorocortisone hemisuccinate.

It contains suitable and harmless buffers, dispersants, irrigants, and preservatives. It is sterile. Its pH is not less than 5.0 nor more than 7.5. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (ii), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, crystallinity, residue on ignition, melting point, and identity.

(*c*) The batch for neomycin content, gramicidin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §444.342b(b)(1)(i). The neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in §444.342b(b)(1)(iii). The content of gramicidin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of sample in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985; 59 FR 8398, Feb. 22, 1994]

§444.342d Neomycin sulfate-polymyxin B sulfate ———— ophthalmic suspension (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension in a suitable and harmless aqueous vehicle containing, in each milliliter, neomycin sulfate, polymyxin B sulfate, and other active ingredients in the following amounts:

(i) 3.5 milligrams of neomycin, 16,250 units of polymyxin B, and either 5 milligrams or 15 milligrams of hydrocortisone acetate; or

(ii) 5 milligrams of neomycin, 15,000 units of polymyxin B, and 2.5 milligrams of hydrocortisone; or

(iii) 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 10.0 milligrams of hydrocortisone; or

(iv) 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 5.0 milligrams of prednisolone acetate.

It contains suitable and harmless buffers, dispersants, irrigants, and preservatives. It is sterile. Its pH is not less than 5.0 and not more than 7.0; except if it contains 10 milligrams per milliliter of hydro–cortisone, its pH is not less than 4.1 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1)(i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1)(i), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch for:

(*1*) All tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content*. Proceed as directed in § 444.42a(b)(1) except prepare the sample as follows: Remove an accurately measured representative portion of the sample with a suitable syringe, place into an appropriate volumetric flask to yield a convenient stock solution. Dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0. Further dilute with 0.1 *M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content*. Remove an accurately measured representative portion with a suitable syringe, dilute to a convenient concentration with 10 percent potassium phosphate buffer, pH 6.0. Further dilute to a concentration of 10 units of polymyxin per milliliter with 10 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin per milliliter. Its content of polymyxin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin that it is represented to contain.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH*. Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19045, May 30, 1974, as amended at 42 FR 37975, July 26, 1977; 47 FR 23441, May 28, 1982; 49 FR 5097, Feb. 10, 1984; 49 FR 34351, Aug. 30, 1984; 50 FR 19919, May 13, 1985; 59 FR 8399, Feb. 22, 1994]

§ 444.342e Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

The requirements for certification and the tests and methods of assay for neomycin sulfate ointment and for neomycin sulfate- ———— ointment are described in § 444.542a.

§ 444.342f Neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

The requirements for certification and the tests and methods of assay for neomycin sulfate-gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment are described in § 444.542f.

§ 444.342g Neomycin sulfate-hydrocortisone acetate ophthalmic suspension; neomycin sulfate-prednisolone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate-hydrocortisone acetate ophthalmic suspension is an aqueous suspension containing in each milliliter 3.5 milligrams of neomycin and 5 milligrams or 15 milligrams of hydrocortisone acetate. Neomycin sulfate-prednisolone acetate ophthalmic suspension is an aqueous suspension containing in each milliliter 3.5 milligrams of neomycin and 2.5 milligrams of prednisolone acetate. The vehicle contains one or more suitable and harmless buffers, preservatives, and dispersants. It is sterile. Its

pH is not less than 5.5 and not more than 7.5. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (v), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §444.42a(b)(1), except prepare the sample for assay as follows: Remove 1.0 milliliter with a suitable syringe, place into an appropriate-sized volumetric flask and dilute to volume with 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Make proper estimated dilutions to the prescribed reference concentration with 0.1*M* potassium phosphate buffer, pH 8.0. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steriod prevents solubilization, use 0.25 milliliter in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19045, May 30, 1974, as amended at 39 FR 33666, Sept. 19, 1974; 50 FR 19919, May 13, 1985]

§444.342h Neomycin sulfate-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ophthalmic ointment contains in each gram, neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 10,000 units of polymyxin B with suitable preservatives in a suitable and harmless ointment base. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) except sterility and pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1) of this chapter except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 47 FR 23441, May 28, 1982; 50 FR 19919, May 13, 1985; 55 FR 14969, Apr. 20, 1990]

§ 444.342i Neomycin sulfate-polymyxin B sulfate ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ophthalmic solution is neomycin sulfate and polymyxin B sulfate in a suitable and harmless aqueous vehicle. Each milliliter contains: (i) Neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 10,000 units of polymyxin B; or

(ii) Neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 16,250 units of polymyxin B. It contains suitable and harmless buffers, dispersants, irrigants, and preservatives. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 5.0 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) except sterility and pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) of this chapter

except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10.0 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[39 FR 19045, May 30, 1974, as amended at 39 FR 36472, Oct. 10, 1974; 47 FR 23441, May 28, 1982; 50 FR 19919, May 13, 1985; 55 FR 14969, Apr. 20, 1990; 59 FR 8399, Feb. 22, 1994]

§444.342j Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic suspension is an aqueous suspension containing in each milliliter 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 1.0 milligram of dexamethasone. It contains suitable and harmless buffers, dispersants, irrigants, and preservatives. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 5.2 and not more than 5.8. The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately measured representative portion of the sample into an appropriate-sized volumetric flask with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 0.25 milliliter in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[47 FR 23441, May 28, 1982; 47 FR 25320, June 11, 1982, as amended at 50 FR 19919, May 13, 1985; 55 FR 14969, Apr. 20, 1990; 59 FR 8399, Feb. 22, 1994]

§ 444.342k Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-dexamethasone ophthalmic ointment contains in each gram neomycin sulfate equivalent to 3.5 milligrams of neomycin, polymyxin B sulfate equivalent to 10,000 units of polymyxin B and 1.0 milligram of dexamethasone with suitable preservatives in a suitable and harmless ointment base. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for neomycin content, polymyxin B content, moisture, and metal particles.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of poylmyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in §436.206 of this chapter.

[47 FR 23442, May 28, 1982; 47 FR 25320, June 11, 1982, as amended at 50 FR 19919, May 13, 1985; 55 FR 14969, Apr. 20, 1990]

§444.380 Tobramycin ophthalmic dosage forms.

§444.380a Tobramycin ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin ophthalmic solution contains in each milliliter 3.0 milligrams of tobramycin in a suitable and harmless aqueous vehicle. It contains suitable and harmless buffers, dispersants, preservatives, and tonicity agents. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. It is sterile. Its pH is not less than 7.0 and not more than 8.0. The tobramycin used conforms to the standards prescribed by §444.80(a)(1), except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[46 FR 16681, Mar. 13, 1981; 46 FR 22359, Apr. 17, 1981. Redesignated at 47 FR 7827, Feb. 23, 1982, and amended at 50 FR 19919, May 13, 1985; 59 FR 8399, Feb. 22, 1994]

§ 444.380b Tobramycin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tobramycin ophthalmic ointment contains, in each gram, 3.0 milligrams of tobramycin with a suitable preservative in a suitable and harmless ointment base. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for metal particles. The tobramycin used conforms to the standards prescribed by § 444.80(a)(1), except heavy metals.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(*b*) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water, and shake well. Allow the layers to separate. Remove the distilled water layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the extractives in a suitable volumetric flask and dilute to volume with distilled water. Further dilute an aliquot with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles*. Proceed as directed in § 436.206 of this chapter.

[47 FR 7827, Feb. 23, 1982; 47 FR 16320, Apr. 16, 1982, as amended at 50 FR 19919, May 13, 1985]

§ 444.380c Tobramycin-dexamethasone ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tobramycin-dexamethasone ophthalmic suspension is an aqueous suspension containing, in each milliliter, 3.0 milligrams of tobramycin and

1.0 milligram of dexamethasone in a suitable and harmless aqueous vehicle. It contains suitable and harmless buffers, dispersants, preservatives, and tonicity agents. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its dexamethasone content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of dexamethasone that it is represented to contain. It is sterile. Its pH is not less than 5.0 and not more than 6.0. It passes the identity tests for tobramycin and dexamethasone. The tobramycin used conforms to the standards prescribed by § 444.80(a)(1), except heavy metals. The dexamethasone used conforms to the standards prescribed by the USP XXI.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The dexamethasone used in making the batch for all USP XXI specifications.

(C) The batch for tobramycin potency, dexamethasone content, sterility, pH, tobramycin identity, and dexamethasone identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Tobramycin potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Dexamethasone content.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate of 1.5 milliliters per minute, and an injection volume of 20 microliters. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Mix acetonitrile:water (45:55). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Accurately weigh approximately 25 milligrams of the dexamethasone working standard into a 25-milliliter volumetric flask containing methanol. Shake until dissolved. Dilute to volume with methanol. Further dilute 4.0 milliliters of this solution to 100 milliliters with methanol to obtain a solution containing approximately 40 micrograms of dexamethasone per milliliter. Mix well.

(B) *Sample solutions.* Remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient methanol to obtain a solution containing 40 micrograms of dexamethasone per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) is satisfactory if it is not more than 1.6 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory

if it is greater than 5,500 theoretical plates.

(C) *Resolution*. The resolution (*R*) between the peak for dexamethasone and its nearest eluting impurity is satisfactory if it is not less than 1.1.

(D) *Coefficient of variation*. The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph(b)(2)(ii)(B) of this section should not be changed.

(iv) *Calculations*. Calculate the dexamethasone content of the container as follows:

$$\text{Milligrams of dexamethasone per container} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the dexamethasone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the dexamethasone peak in the chromatogram of the dexamethasone working standard;

P_s=Dexamethasone content in the dexamethasone working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(3) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Tobramycin identity*. Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows; decant 1 milliliter into a test tube. Add 100 milligrams of sodium sulfate to the test tube and shake until the sodium sulfate has been dispersed. Centrifuge to obtain a clear supernatant. Use the supernatant as the sample solution.

(6) *Dexamethasone identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the dexamethasone working standard.

[54 FR 13879, Apr. 6, 1989, as amended at 59 FR 8399, Feb. 22, 1994]

§ 444.380d Tobramycin-dexamethasone ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tobramycin-dexamethasone ophthalmic ointment contains in each gram, tobramycin equivalent to 3.0 milligrams of tobramycin and 1.0 milligram of dexamethasone, with a suitable preservative in a suitable and harmless white petrolatum base. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its dexamethasone content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of dexamethasone that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for metal particles. It passes the identity tests for tobramycin and dexamethasone. The tobramycin used conforms to the standards prescribed by § 444.80(a)(1), except heavy metals. The dexamethasone used conforms to the standards prescribed by the U.S. Pharmacopeia XXII.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The dexamethasone used in making the batch for all U.S. Pharmacopeia XXII specifications.

(C) The batch for tobramycin potency, dexamethasone content, sterility, moisture, metal particles, tobramycin identity, and dexamethasone identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Tobramycin potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the distilled water layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the extractives in a suitable volumetric flask and dilute to volume with distilled water. Further dilute an aliquot with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Dexamethasone content*. Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate of 1.5 milliliters per minute, and an injection volume of 20 microliters. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Mix acetonitrile:water (45:55) and adjust if necessary by reducing the amount of acetonitrile to increase retention, or by increasing the amount of acetonitrile to decrease the retention of the solute. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions and resolution test solution*—(A) *Working standard solution*. Accurately weigh approximately 20 milligrams of the dexamethasone working standard into a 100-milliliter volumetric flask containing methonol:water (75:25). Shake until dissolved. Dilute to volume with methanol:water (75:25). Transfer 10.0 milliliters of this solution to a separatory funnel containing approximately 50 milliliters of hexane. Shake until homogeneous. Add 15.0 milliliters of methanol:water (75:25) and shake well. Allow the layers to separate. Remove the lower (methanol:water) layer and repeat the extraction twice more with 15.0 milliliters of methanol:water (75:25). Collect the extractives in a 50-milliliter volumetric flask. Dilute to volume with methanol:water (75:25) to obtain a solution of known concentration containing approximately 40 micrograms of dexamethasone per milliliter.

(B) *Sample solution*. Accurately weigh approximately 2.0 grams of the sample and place into a separatory funnel containing approximately 50 milliliters of hexane. Shake until homogeneous. Add 15.0 milliliters of methanol:water (75:25) and shake well. Allow the layers to separate. Remove the lower (methanol:water) layer and repeat the extraction twice more with 15.0 milliliters of methanol:water (75:25). Collect the extractives in a 50-milliliter volumetric flask. Dilute to volume with methanol:water (75:25) to obtain a solution of known concentration containing approximately 40 micrograms of dexamethasone per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Asymmetry*. The asymmetry (A_s) is satisfactory if it is not more than 1.6 at 10 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (n) is satisfactory if it is greater than 5,500 theoretical plates.

(C) *Resolution*. The resolution (R_s) between the peak for dexamethasone and its nearest eluting impurity is satisfactory if it is not less than 1.1.

(D) *Coefficient of variation*. The coefficient of variation (*RSD* in percent) of 5 replicate injections is satisfactory if it

is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the dexamethasone content as follows:

$$\text{Milligrams of dexamethasone per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the dexamethasone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the dexamethasone peak in the chromatogram of the dexamethasone working standard;

P_s=Dexamethasone content in the dexamethasone working standard solution in micrograms per milliliter;

d=Dilution factor of the sample; and

n=Number of grams of sample assayed.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

(6) *Tobramycin identity.* Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows: Weigh approximately 1 gram of the sample into a test tube. Add 1 to 2 milliliters of chloroform to the test tube and shake vigorously to dissolve the ointment. Centrifuge for approximately 15 minutes to clearly separate the layers. Use the top (aqueous) layer in the procedure.

NOTE: If an oily film remains on the top of the aqueous layer and interferes with sampling, the aqueous layer may be transferred to another test tube and washed with an additional 1 to 2 milliliters of chloroform.

(7) *Dexamethasone identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the dexamethasone working standard.

[55 FR 617, Jan. 8, 1990]

§ 444.380e Tobramycin-fluorometholone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin-fluorometholone acetate ophthalmic suspension is an aqueous suspension containing, in each milliliter, 3.0 milligrams of tobramycin and 1.0 milligram of fluorometholone acetate in a suitable and harmless aqueous vehicle. It contains one or more suitable and harmless dispersants, preservatives, buffers, and tonicity agents. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its fluorometholone acetate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of fluorometholone acetate than it is represented to contain. It is sterile. Its pH is not less than 6.0 and not more than 7.0. It passes the identity tests for tobramycin and fluorometholone acetate. The tobramycin used conforms to the standards prescribed by § 440.80(a)(1) of this chapter, except heavy metals. The fluorometholone acetate used conforms to the standards prescribed in the U.S. Pharmacopeia XXII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The fluorometholone acetate used in making the batch for all requirements in U.S. Pharmacopeia XXII.

(C) The batch for tobramycin potency, fluorometholone acetate content, sterility, pH, tobramycin identity, and fluorometholone acetate identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Tobramycin potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Fluorometholone acetate content*. Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column or cartridge packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and an injection volume of 10 or 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Mix acetonitrile:water (50:50) and adjust if necessary by reducing the amount of acetonitrile to increase retention, or by increasing the amount of acetonitrile to decrease the retention of the solute. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions, and resolution test solution*—(A) *Working standard solution*. Accurately weigh approximately 25 milligrams of the fluorometholone acetate working standard into a 10-milliliter volumetric flask and add about 5 milliliters of acetonitrile. Shake until dissolved. Dilute to volume with acetonitrile. Further dilute 1.0 milliliter of this solution in a volumetric flask to 10 milliliters with acetonitrile to obtain a solution of known concentration containing approximately 250 micrograms of fluorometholone acetate per milliliter. Mix well.

(B) *Sample solution*. Shake vial thoroughly, to homogenize its contents, and immediately remove an accurately measured representative portion from it. Quantitatively dilute the suspension thus obtained with sufficient acetonitrile to obtain a solution containing 250 micrograms of fluorometholone acetate per milliliter (estimated). For instance, dilute a 1.0 milliliter aliquot of suspension with 3.0 milliliters of acetonitrile and filter.

(C) *Resolution test solution*. Prepare as directed in paragraph (b)(2)(ii)(A) of this section, except use 10 milligrams of fluorometholone in addition to the 25 milligrams of fluorometholone acetate working standard.

(iii) *System suitability requirements*—(A) *Asymmetry*. The asymmetry (A_S) is satisfactory if it is not more than 1.35 at 10 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (h_r) is satisfactory if it is not greater than 20, equivalent to 1,000 plates for a 10-centimeter column of 5 microns or 2,500 plates for a 25-centimeter column of 5 micron size particles.

(C) *Resolution*. The resolution (R_S) between the peaks of fluorometholone acetate and fluorometholone is satisfactory if it is not less than 2.0.

(D) *Capacity factor*. The capacity factor (k) for fluorometholone acetate is satisfactory if it is in the range between 1.0 and 5.0.

(E) *Coefficient of variation*. The coefficient of variation (*RSD* in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the fluorometholone acetate content of the container as follows:

$$\text{Milligrams of fluorometholone acetate per container} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_U=Area of the fluorometholone acetate peak in the chromatogram of the sample

(at a retention time equal to that observed for the standard);

A_S=Area of the fluorometholone acetate peak in the chromatogram of the fluorometholone acetate working standard;

P_S=Fluorometholone acetate content in the fluorometholone acetate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(3) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

(5) *Tobramycin identity*. Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows: Decant 1.0 milliliter of the unshaken sample into a test tube. Add 100 milligrams of sodium sulfate to the test tube and shake until the sodium sulfate has been dispersed. Centrifuge to obtain a clear supernatant. Use the supernatant as the sample solution.

(6) *Fluorometholone acetate identity*. The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the fluorometholone acetate working standard.

[58 FR 26671, May 4, 1993]

Subpart E—Otic Dosage Forms

§ 444.442 Neomycin sulfate otic dosage forms.

§§ 444.442a—444.442c [Reserved]

§ 444.442d Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

The requirements for certification and the tests and methods of assay for neomycin sulfate ointment and for neomycin sulfate- ———— ointment are described in § 444.542a.

§ 444.442e [Reserved]

§ 444.442f Neomycin sulfate-hydrocortisone-acetic acid otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate-hydrocortisone-acetic acid otic suspension is an aqueous suspension containing in each milliliter 5.0 milligrams of neomycin sulfate equivalent to 3.5 milligrams of neomycin and 10 milligrams of hydrocortisone. It also contains 2 percent acetic acid. It may contain one or more suitable and harmless buffers, preservatives, and dispersants. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. It is sterile. Its pH is not less than 4.5 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed in § 444.42a(a)(1)(i), (v), (vi), and (vii).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The samples used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove an accurately measured representative portion of the sample and dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter in lieu

of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[39 FR 33668, Sept. 19, 1974, as amended at 46 FR 25608, May 8, 1981]

§ 444.442g Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic suspension contains in each milliliter 3.5 milligrams neomycin, 10,000 units polymyxin B, and 10 milligrams hydrocortisone in a suitable and harmless vehicle. It may also contain one or more suitable and harmless buffers, dispersants, and preservatives. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 3.0 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Dilute an accurately measured representative portion with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents in lieu of 1 milliliter and proceed as disolubilization, use 0.25 milliliter of sample as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[40 FR 22252, May 22, 1975, as amended at 50 FR 19919, May 13, 1985; 55 FR 18598, May 3, 1990]

§ 444.442h Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-hydrocortisone otic solution contains in each milliliter 3.5 milligrams of neomycin, 10,000 units of

polymyxin B, and 10 milligrams of hydrocortisone in a suitable and harmless vehicle. It may also contain one or more suitable and harmless buffers, dispersants, and solvents. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. The pH is not less than 2.0 and not more than 4.5. The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for neomycin content, polymyxin B content, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, except add to each concentration of polymyxin B standard response line a quantity of neomycin equal to the amount present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[41 FR 14186, Apr. 2, 1976; 46 FR 55091, Nov. 6, 1981, as amended at 50 FR 19919, May 13, 1985]

Subpart F—Dermatologic Dosage Forms

§444.520 Gentamicin sulfate dermatologic dosage forms.

§444.520a Gentamicin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate ointment is gentamicin sulfate with suitable preservatives in a white petrolatum base. Each gram contains gentamicin sulfate equivalent to 1.0 milligram of gentamicin. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin that it is represented to contain. Its moisture content is not more than 1.0 percent. The gentamicin sulfate used conforms to the standards prescribed therefor by §444.20(a)(1).

(2) *Packaging.* In addition to the requirements of §432.1 of this chapter, it may be dispensed from a pressurized container wherein it is maintained in a compartment separate from the gas used to supply the pressure.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency and moisture.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous, add 20–25 milliliters of 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer and repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.520b Gentamicin sulfate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate cream is gentamicin sulfate with one or more suitable emollients, dispersants, and preservatives in a suitable and harmless cream base. Each gram contains gentamicin sulfate equivalent to 1.0 milligram of gentamicin. Its potency is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of gentamicin that it is represented to contain. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20(a)(1).

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, it may be dispensed from a pressurized container wherein it is maintained in a compartment separate from the gas used to supply the pressure.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, gentamicins C_1, C_{1a}, and C_2, and identity.

(*b*) The batch for gentamicin potency.

(ii) Samples required:

(*a*) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the cream into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, ph 8.0 (solution 3), and shake gently to avoid gel formation. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20 to 25 milliliter quantities of solution 3. Combine the buffer extractives and adjust to an appropriate volume to obtain a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration

of 0.1 microgram of gentamicin per milliliter (estimated).

[39 FR 19046, May 30, 1974, as amended at 46 FR 45332, Sept. 11, 1981; 50 FR 19919, May 13, 1985]

§ 444.540 Neomycin palmitate dermatologic dosage forms.

§ 444.542 Neomycin sulfate dermatologic dosage forms.

§ 444.542a Neomycin sulfate ointment; neomycin sulfate- ———— ointment (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate ointment contains, in each gram, 3.5 milligrams of neomycin in a suitable and harmless water-soluble or oleaginous ointment base, with or without one or more suitable and harmless dispersants, emollients, and preservatives. The following other drugs may be combined with neomycin sulfate ointment in the indicated amounts, per gram:

(i) If it is for topical use:

(*a*) 0.5 milligram of flurandrenolide; or

(*b*) 0.25 milligram of fluorometholone; or

(*c*) 5.0, 10.0, 15.0, or 25.0 milligrams of hydrocortisone acetate; or

(*d*) 10.0 or 25.0 milligrams of hydrocortisone; or

(*e*) 5.0 milligrams of hydrocortamate hydrochloride; or

(*f*) 1.0, 2.5, or 5.0 milligrams of prednisolone acetate; or

(*g*) 1.0 milligram of triamcinolone acetonide; or

(*h*)–(*i*) [Reserved]

(*j*) 200 milligrams of benzocaine.

(ii) If it is for ophthalmic use:

(*a*) 5.0 milligrams or 15.0 milligrams of hydrocortisone acetate; or

(*b*) 2.5 milligrams of sodium prednisolone phosphate; or

(*c*) 0.5 milligram of sodium dexamethasone phosphate.

(*d*) 1.0 milligram of methyprednisolone; or

(*e*) 1.0 milligram of triamcinolone acetonide; or

(*f*) 2.5 milligrams or 5.0 milligrams of prednisolone acetate; or

(*g*) 15.0 milligrans of cortisone acetate.

If it is an oleaginous base, its moisture content is not more than 1.0 percent. If it is intended for ophthalmic use, it is sterile. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a steroid or if it is intended for ophthalmic use, it shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter, and its expiration date is 12 months. If it does not contain a steroid or it is not intended for ophthalmic use each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency and for moisture if the ointment base is oleaginous and for sterility if the ointment is intended for ophthalmic use.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Extraction*. Proceed as directed in §444.42a(b)(1) of this chapter, except prepare the sample by placing an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration.

(ii) *Blending*. Proceed as directed in §444.42a(b)(1), except prepare the sample for assay as follows: Transfer an accurately weighed sample into a high-speed glass blender, add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility*. If the ointment is intended for ophthalmic use, proceed as directed in §436.20 of this chapter, using the method as described in paragraph (e)(3) of that section.

(3) *Moisture*. If the ointment has an oleaginous base, proceed as directed in §436.201 of this chapter.

[39 FR 19045, May 30, 1974, as amended at 39 FR 33666, Sept. 19, 1974; 47 FR 23442, May 28, 1982; 49 FR 34351, Aug. 30, 1984; 50 FR 19919, May 13, 1985; 50 FR 47213, Nov. 15, 1985]

§444.542b Neomycin sulfate cream; neomycin sulfate_____ cream (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Neomycin sulfate cream contains, in each gram, 3.5 milligrams of neomycin in a suitable cream base, with or without one or more suitable and harmless emollients, perfumes, dispersants, and preservatives. The following other drugs may be combined with neomycin sulfate cream in the indicated amounts per gram:

(i) 2 milligrams of betamethasone; or

(ii) Dexamethasone sodium phosphate equivalent to 1.0 milligram of dexamethasone phosphate; or

(iii) 1 milligram of sodium dexamethasone phosphate; or

(iv) 2.5 milligrams of dichlorisone acetate; or

(v) 0.25 milligram of fluocinolone acetonide; or

(vi) 2.5 milligrams, 5 milligrams, or 10 milligrams of methylprednisolone acetate; or

(vii) 1 milligram of triamcinolone acetonide; or

(viii) 2.5 milligrams, 5.0 milligrams, or 10.0 milligrams of hydrocortisone; or

(ix) 10.0 milligrams or 25.0 milligrams of hydrocortisone acetate; or

(x) 0.5 milligram of flurandrenolide.

The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling*. If it contains a corticosteroid, it shall be labeled in accordance with the requirements prescribed by §432.5 of this chapter, and its expiration date is 12 months. If it does not contain a corticosteriod, each

package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 444.42a(b)(1), except prepare the sample for assay as follows: Transfer an accurately weighed representative portion into a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration and blend 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. Its neomycin content is satisfactory if it is not less than 90 percent nor more than 135 percent of the number of milligrams of neomycin that it is represented to contain.

[39 FR 19046, May 30, 1974, as amended at 49 FR 34351, Aug. 30, 1984; 53 FR 18838, May 25, 1988]

§ 444.542c Neomycin sulfate-_____ lotion (the blank being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension containing, in each milliliter, 3.5 milligrams of neomycin and the following other active ingredients in a suitable and harmless vehicle:

(i) 10 milligrams of diperodon hydrochloride and 7.5 milligrams of aluminum dihydroxy allantoinate; or

(ii) 5 milligrams or 10 milligrams of hydrocortisone acetate; or

(iii) 5 milligrams, 10 milligrams, or 20 milligrams of hydrocortisone; or

(iv) 1 milligram, 2.5 milligrams, or 5 milligrams of prednisolone acetate; or

(v) Prednisolone sodium phosphate equivalent to 5.0 milligrams of prednisolone phosphate; or

(vi) 0.5 milligram of flurandrenolide.

It may also contain one or more suitable and harmless dispersants, emollients, and preservatives. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a corticosteroid, it shall be labeled in accordance with the requirements prescribed by § 432.5 of this chapter and its expiration date is 12 months. If it does not contain a corticosteroid, each package shall bear, on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency.* Proceed as directed in §444.42a(b)(1), except prepare the sample for assay as follows: Place an accurately measured representative portion into a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make further dilutions with 0.1*M* potassium phosphate buffer, pH 8, to the proper prescribed reference concentration. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

[39 FR 19046, May 30, 1974, as amended at 49 FR 34351, Aug. 30, 1984]

§444.542d [Reserved]

§444.542e Neomycin sulfate–polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ointment is an ointment containing, in each gram, 3.5 milligrams of neomycin and 5,000 units of polymyxin B with suitable and harmless emollients, dispersants, and preservatives in a suitable and harmless water-miscible base. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1) (i), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content and polymyxin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package

of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.542a(b)(1)(ii). Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin standards response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Remove the buffer layer and repeat the extraction procedure with each of three more 20 to 25 milliliter quantities of solution 6. Combine the extractives in a suitable volumetric flask and fill to volume with solution 6. Further dilute an aliquot with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated). Its content of polymyxin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin that it is represented to contain.

§ 444.542f Neomycin sulfate–gramicidin topical ointment; neomycin sulfate-gramicidin-triamcinolone acetonide ointment; neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each gram of neomycin sulfate-gramicidin topical ointment contains 2.5 milligrams of neomycin and 0.25 milligram of gramicidin. Neomycin sulfate-gramicidin-triamcinolone acetonide ointment is an ointment containing, in each gram, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetonide. Neomycin sulfate-gramicidin-fludrocortisone acetate ointment is an ointment containing, in each gram, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of fludrocortisone acetate. If it is intended for ophthalmic use, it is sterile. Their moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (v), (vi), and (vii), and in addition if it is used in the preparation of an ophthalmic ointment, paragraph (a)(1) of that section. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter, and in addition if it is used in the preparation of an ophthalmic ointment, paragraph (a)(1) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* If it contains a steroid or it is intended for ophthalmic use, it shall be labeled in accordance with the requirements of § 432.5 of this chapter, and its expiration date is 12 months. If it does not contain a steroid or it is not intended for ophthalmic use, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certifications; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, crystallinity, and identity.

(*c*) The batch for neomycin content, gramicidin content, and moisture, and for sterility if it is intended for ophthalmic use.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §444.542a(b)(1). Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in §448.25(b)(1) of this chapter, except prepare the sample for assay by the following method: Place an accurately weighed representative portion into a separatory funnel. Dissolve the ointment in approximately 50 milliliters of petroleum ether. Extract this solution with four 20-milliliter portions of 80 percent alcohol prepared from alcohol U.S.P. XX. Combine the extractives in a suitable volumetric flask, bring to volume with alcohol U.S.P. XX, and mix well. From this stock solution, dilute an aliquot with alcohol U.S.P. XX to the reference concentration. Its content of gramicidin is satisfactory if it is not less than 90 percent and not more that 140 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams of solids.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 47 FR 23709, June 1, 1982; 50 FR 19919, May 13, 1985]

§444.542g Neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin-triamcinolone acetonide cream is a cream containing 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetonide per gram, with one or more suitable and harmless emollients, dispersants, and preservatives in a suitable and harmless cream base. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by §448.25(a)(1) (i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., shall conform to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by §432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, residue on ignition, melting point, crystallinity and identity.

(*c*) The batch for neomycin content and gramicidin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay; potency*—(1) *Neomycin content.* Proceed as directed in § 444.542(b). Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except to prepare the sample for assay proceed as follows: Place an accurately weighed representative portion into a high-speed glass blender jar and add sufficient alcohol U.S.P. XX to obtain a stock solution of convenient concentration. Blend 3 to 5 minutes. Make proper estimated dilutions of an aliquot to the reference concentration with alcohol U.S.P. XX. Its content of gramicidin is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

[39 FR 19045, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 47 FR 23709, June 1, 1982]

§ 444.542h Neomycin sulfate-gramicidin-triamcinolone acetonide lotion; neomycin sulfate-gramicidin-fludrocortisone acetate lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* The drug is a lotion containing, in each milliliter, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and either 1 milligram of triamcinolone acetonide or 1 milligram of fludrocortisone acetate, with one or more suitable and harmless emollients, buffers, dispersants, and preservatives, in a suitable and harmless lotion base. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1)(i), (vi), and (vii). The gramicidin used conforms to the standards prescribed by § 448.25(a)(1)(i), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, crystallinity, residue on ignition, melting point, and identity.

(*c*) The batch for neomycin content and gramicidin content.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 444.542c(b). Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 448.25(b)(1) of this chapter, except prepare the sample by placing an accurately measured representative portion into a high-speed glass blender jar with sufficient alcohol U.S.P. XX to obtain a stock solution of convenience concentration. Blend 3 to 5 minutes.

Make proper estimated dilutions in alcohol U.S.P. XX to the reference concentration. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain.

[39 FR 19045, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 47 FR 23709, June 1, 1982]

§444.542i [Reserved]

§444.542j Neomycin sulfate-polymyxin B sulfate-gramicidin-benzocaine ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-gramicidin-benzocaine ointment is neomycin sulfate, polymyxin B sulfate, gramicidin, and benzocaine, with suitable and harmless preservatives, in white petrolatum. Each gram contains 3.5 milligrams of neomycin, 2,000 units of polymyxin B, 0.25 milligram of gramicidin, and 10 milligrams of benzocaine. The moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (v), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1) (i), (v), (vi), (vii), and (ix) of this chapter. The gramicidin used conforms to the standards prescribed by §448.25(a)(1)(i), (iii), (iv), (v), and (vi) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that is 12 months after the month during which the batch was certified.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, residue on ignition, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for neomycin content, polymyxin B content, gramicidin content, and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §444.542a(b)(1)(i) or (ii). The content of neomycin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in §444.542e(b)(1)(ii) of this chapter. The content of polymyxin B is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in §448.25(b)(1) of this chapter, except prepare the sample for assay as follows: Place approximately 1 gram of

the ointment, accurately weighed, into a high-speed glass blender. Add that quantity of alcohol U.S.P. XX which is sufficient to obtain a stock solution of convenient concentration. Blend 3 to 5 minutes. Make proper estimated dilutions of an aliquot to the reference concentration with alcohol U.S.P. XX. The content of gramicidin is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 47 FR 23710, June 1, 1982]

§ 444.542k Neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-hydrocortisone acetate cream contains, in each gram, neomycin sulfate equivalent to 3.5 milligrams of neomycin, polymyxin B sulfate equivalent to 10,000 units of polymyxin B, and 5.0 milligrams of hydrocortisone acetate in a suitable and harmless vehicle. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identify.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for neomycin content and polymyxin B content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay; potency*—(1) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Transfer an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Transfer an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

[50 FR 15108, Apr. 17, 1985, as amended at 55 FR 11584, Mar. 29, 1990]

§444.542l Neomycin sulfate-polymyxin B sulfate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate cream is a cream containing, in each gram, neomycin sulfate equivalent to 3.5 milligrams of neomycin and polymyxin B sulfate equivalent to 10,000 units of polymyxin B in a suitable and harmless vehicle. It may contain a suitable local anesthetic. Its neomycin sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1) of this chapter.

(2) *Labeling*—(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark;

(*b*) The name and quantity of each active ingredient contained in the drug; and

(*c*) An expiration date that conforms to the requirements prescribed by §432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for neomycin content and polymyxin B content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay; potency*—(1) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Transfer an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Transfer an accurately weighed portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

[50 FR 15109, Apr. 17, 1985, as amended at 55 FR 11584, Mar. 29, 1990; 55 FR 50173, Dec. 5, 1990]

Subparts G–I [Reserved]

Subpart J—Certain Other Dosage Forms

§ 444.942 Neomycin sulfate in certain other dosage forms.

§ 444.942a Neomycin sulfate for compounding oral products.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate for compounding oral products is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH is an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 nor more than 7.5.

(v) It gives a positive identity test for neomycin.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain not less than 10 grams and not more than 100 grams of neomycin sulfate.

(3) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 432.5(a) of this chapter. Its expiration date is 12 months.

(4) *Requests for certification; samples.* (i) In addition to complying with the conditions of § 431.1 of this chapter, a person who requests certification of a batch of neomycin sulfate for compounding oral products shall submit with the request a statement showing the batch mark, the number of packages of each size in the batch, and the date on which the latest assay of the drug comprising such batch was completed. Such request shall be accompanied or followed by results of tests and assays made on the batch for potency, moisture, pH, and identity.

(ii) Such person shall submit with his request a sample consisting of a 0.5 gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay; potency, moisture, pH, and identity.* Proceed as directed in § 444.42a(b) (1), (5), (6), and (7).

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985; 53 FR 12658, Apr. 15, 1988; 53 FR 31837, Aug. 22, 1988]

§ 444.942b Sterile neomycin sulfate and polymyxin B sulfate solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile neomycin sulfate and polymyxin B sulfate solution is an aqueous solution containing in each milliliter 40 milligrams of neomycin and 200,000 units of polymyxin B. If packaged in a multiple-dose container, it shall contain a suitable and harmless preservative. It is sterile. Its pH is not less than 4.5 and not more than 6.0, except that for issuance of a certificate it is not less than 5.0. The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) (i), (vi), and (vii). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) (i), (vi), (vii), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or the N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to being labeled in accordance with the requirements of § 432.5 of this chapter, the labeling shall include a statement to the effect that the drug is to be diluted for use as a urinary bladder irrigant and is not for injection. Its expiration date is 12 months.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, residue on ignition, and identity.

(*c*) The batch for neomycin content, polymyxin B content, pH, and sterility.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: Ten packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: Ten packages, each containing approximately 300 milligrams

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in §444.42a(b)(1), except prepare the sample as follows: Remove an accurately measured portion and dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to the proper prescribed reference concentration. The neomycin content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of milligrams of neomycin that it is represented to contain.

(ii) *Polymyxin B content.* Remove an accurately measured portion and dilute with 10-percent potassium phosphate buffer, pH 6.0, to a reference concentration of 10 units of polymyxin B per milliliter. Proceed as directed in §448.30a(b)(1) of this chapter, except add to each concentration of the polymyxin B standard curve a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. The polymyxin B content is satisfactory if it is not less than 90 percent nor more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19045, May 30, 1974, as amended at 41 FR 56307, Dec. 28, 1976; 42 FR 18059, Apr. 5, 1977; 50 FR 19919, May 13, 1985]

PART 446—TETRACYCLINE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
446.10 Chlortetracycline hydrochloride.
446.10a Sterile chlortetracycline hydrochloride.
446.15 Demeclocycline.
446.16 Demeclocycline hydrochloride.
446.20 Doxycycline hyclate.
446.20a Sterile doxycycline hyclate.
446.21 Doxycycline monohydrate.
446.42 Meclocycline sulfosalicylate.
446.50 Methacycline hydrochloride.
446.60 Minocycline hydrochloride.
446.65 Oxytetracycline.
446.65a Sterile oxytetracycline.
446.66 Oxytetracycline calcium.
446.67 Oxytetracycline hydrochloride.
446.67a Sterile oxytetracycline hydrochloride.
446.75a Sterile rolitetracycline.
446.76a Sterile rolitetracycline nitrate.
446.80 Tetracycline.
446.81 Tetracycline hydrochloride.
446.81a Sterile tetracycline hydrochloride.
446.82 Tetracycline phosphate complex.

Subpart B—Oral Dosage Forms

446.110 Chlortetracycline hydrochloride capsules.
446.115 Demeclocycline oral dosage forms.
446.115a Demeclocycline oral suspension.
446.115b Demeclocycline for oral suspension.
446.116 Demeclocyline hydrochloride oral dosage forms.
446.116a Demeclocycline hydrochloride tablets.
446.116b [Reserved]
446.116c Demeclocycline hydrochloride capsules.
446.120 Doxycycline hyclate oral dosage forms.
446.120a Doxycycline hyclate capsules.
446.120b Doxycycline calcium oral suspension.
446.120c Doxycycline hyclate tablets.
446.120d Doxycycline hyclate pellet–filled capsules.
446.121 Doxycycline monohydrate oral dosage forms.
446.121a Doxycycline monohydrate for oral suspension.
446.121b Doxycycline monohydrate capsules.
446.150 Methacycline hydrochloride oral dosage forms.
446.150a Methacycline hydrochloride capsules.
446.150b Methacycline hydrochloride oral suspension.
446.160 Minocycline hydrochloride oral dosage forms.
446.160a Minocycline hydrochloride tablets.

446.160b Minocycline hydrochloride capsules.
446.160c Minocycline hydrochloride oral suspension.
446.165 Oxytetracycline oral dosage forms.
446.165a Oxytetracycline tablets.
446.165b—446.165c [Reserved]
446.165d Oxytetracycline for oral suspension.
446.166 Oxytetracycline calcium oral suspension.
446.167 Oxytetracycline hydrochloride capsules.
446.180 Tetracycline oral dosage forms.
446.180a—446.180b [Reserved]
446.180c Tetracycline oral suspension.
446.181 Tetracycline hydrochloride oral dosage forms.
446.181a—446.181c [Reserved]
446.181d Tetracycline hydrochloride tablets.
446.181e Tetracycline hydrochloride capsules.
446.182 Tetracycline phosphate complex capsules.

Subpart C—Injectable Dosage Forms

446.220 Doxycycline hyclate for injection.
446.260 Sterile minocycline hydrochloride.
446.265 Oxytetracycline injection.
446.267 Oxytetracycline hydrochloride for injection.
446.275 Rolitetracycline injectable dosage forms.
446.275a Rolitetracycline for intravenous use.
446.275b Rolitetracycline for intramuscular use.
446.276 Rolitetracycline nitrate injectable dosage forms.
446.276a Rolitetracycline nitrate for intravenous use.
446.276b Rolitetracycline nitrate for intramuscular use.
446.281 Tetracycline hydrochloride injectable dosage forms.
446.281a Sterile tetracycline hydrochloride.
446.281c Tetracycline hydrochloride for intramuscular use.
446.281d Tetracycline hydrochloride for intravenous use.
446.282 Tetracycline phosphate complex for injection.

Subpart D—Ophthalmic Dosage Forms

446.310 Chlortetracycline hydrochloride ophthalmic ointment.
446.367 Oxytetracycline hydrochloride ophthalmic dosage forms.
446.367c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic suspension.
446.367e Oxytetracycline hydrochloride-polymyxin B sulfate ophthalmic ointment.
446.381 Tetracycline hydrochloride ophthalmic dosage forms.
446.381a Tetracycline hydrochloride ophthalmic ointment.
446.381b Tetracycline hydrochloride ophthalmic suspension.

Subpart E—Otic Dosage Forms

446.467 Oxytetracycline hydrochloride-polymyxin B sulfate otic ointment.

Subpart F—Dermatologic Dosage Forms

446.510 Chlortetracycline hydrochloride ointment.
446.542 Meclocycline sulfosalicylate cream.
446.567 Oxytetracycline hydrochloride dermatologic dosage forms.
446.567a [Reserved]
446.567b Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.
446.567c Oxytetracycline hydrochloride-polymyxin B sulfate topical powder.
446.581 Tetracycline hydrochloride dermatologic dosage forms.
446.581a—446.581b [Reserved]
446.581c Tetracycline hydrochloride for topical solution.
446.581d Tetracycline hydrochloride ointment.

Subpart G—Vaginal Dosage Forms

446.667 Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets.

Subpart H—Rectal Dosage Forms [Reserved]

Subpart I [Reserved]

Subpart J—Certain Other Dosage Forms [Reserved]

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19076, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§ 446.10 Chlortetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride is [4S - (4α,4aα,5aα,6β, 12aα] - 7 - chloro - 4 - (dimethylamino) - 1,4, 4a,5,5a,6,11,12a - octahydro - 3,6,10,12, 12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamidemonohydrochloride. Chlortetracycline is produced by the growth of *Streptomyces*

aureofaciens. It is a yellow powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 3.3.

(v) It is crystalline.

(vi) It meets the identity test for chlortetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.01*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of chlortetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(6) *Identity.* To 1 milligram of sample, add 2.0 milliliters of concentrated sulfuric acid. In the presence of chlortetracycline, a deep blue color is produced that becomes dark green.

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.10a Sterile chlortetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride is [4S - (4α,4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. Chlortetracycline is produced by the growth of *Streptomyces aureofaciens.* It is a yellow powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 3.3.

(viii) It is crystalline.

(ix) It meets the identity test for chlortetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, crystallinity, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.01*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of chlortetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with

sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of chlortetracycline hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* To 1.0 milligram of sample, add 2.0 milliliters of concentrated sulfuric acid. In the presence of chlortetracycline, a deep blue color is produced that becomes dark green.

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.15 Demeclocycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline is [4S - (4α,4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,6,10,12,12a - pentahydroxy - 1, 11 - dioxo - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of demeclocycline hydrochloride equivalent per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 6.7 percent.

(iv) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 4 and not more than 5.5.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the demeclocycline hydrochloride working standard is 107.4±3.88.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10-milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the demeclocycline hydrochloride

working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{Weight of sample in milligrams} \times (100 - m)}$$

where: m=percent moisture in the sample.

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity*. Proceed as directed in § 446.16(b)(7). The value yielded by calculation ranges between 0.97 and 1.17.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 16683, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

§ 446.16 Demeclocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Demeclocycline hydrochloride is [4S - (4α 4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro-3,6,10,12,12a - pentahydroxy - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2 and not more than 3.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the demeclocycline hydrochloride standard is 100±4.2 percent.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity*. Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10 milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of

the working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{Weight of sample in milligrams} \times (100 - m)}$$

where: m=percent moisture in the sample.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Accurately weigh 40 milligrams of the sample and place into a 200-milliliter volumetric flask. Add 100 milliliters of 0.1*N* HCl and place on a shaker until the sample is dissolved. Dilute to volume with 0.1*N* HCl and mix well. Transfer a 5-milliliter aliquot of the solution to each of two 50-milliliter volumetric flasks. To one flask add 10 milliliters of 6*N* HCl and to the other add 10 milliliters of 3*N* HCl. Place the acid-treated flasks into a boiling water batch for 20 minutes. Remove the flasks and place in a cold water bath. When cool, dilute to volume with water and mix well. Treat a portion of the standard in the same manner. Using a suitable spectrophotometer, place the 6*N* HCl-treated sample into the reference cell and read against the 3*N* HCl-treated sample at a wavelength of 368 nanometers. Reverse the order of the cells in the cell holder and read at a wavelength of 430 nanometers.

$$\frac{(A_{368} + A_{430}\ \text{sample})\ (\text{milligrams of standard per milliliter})\ (100)}{(A_{368} + A_{430}\ \text{standard})\ (\text{milligrams of sample per milliliter})\ (100 - m)} = 0.9 \text{ to } 1.1$$

where: m=percent moisture in the sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 50 FR 19920, May 13, 1985]

§ 446.20 Doxycycline hyclate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate is [4S-4aα,4aα,5aα,5aα,6α,12aα]-4-dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide hydrochloridehemiethanolate hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) It contains not less than 82 nor more than 90 percent doxycycline on an ''as is'' basis.

(vi) It gives a positive identity test for doxycycline hyclate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content*—(i) *Equipment*—(*a*) *Sheet* (*chromatographic*). Whatman No. 4 filter paper for chromatography, 15 × 57 centimeters.

(*b*) *Chamber* (*chromatographic*). Square glass chromatography jar, 30 × 30 × 60 centimeters, equipped with 25-centimeter troughs for descending chromatography.

(ii) *Preparation of solutions*—(*a*) *0.05N Methanolic hydrochloric acid*. Dilute 4.2 milliliters of concentrated hydrochloric acid to 1 liter with methanol.

(*b*) *pH 4.2 buffer*. Mix 5.86 volumes of 0.1*M* citric acid with 4.14 volumes of 0.2*M* disodium phosphate.

(*c*) *Chromatographic system*. Mix toluene, pyridine, and pH 4.2 buffer in volumetric proportions of 20:3:10, respectively. Allow the phases to separate. Place the upper phase in the troughs near the top of the chamber. Place the lower phase in the bottom of the chamber. Saturate the atmosphere of the tightly sealed chamber for 24 hours before use by placing white blotters on two opposite sides of the chamber so that their ends are immersed in the lower phase in the bottom of the chamber. Replace the solvent in troughs before the chromatograms are to be developed.

(iii) *Preparation of the doxycycline standard solution*. Accurately weigh about 50 milligrams of the doxycycline working standard into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid. Store in the refrigerator and use within 7 days.

(iv) *Preparation of sample*. Accurately weight about 50 milligrams of the sample into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid.

(v) *Preparation of the chromatogram*. Dip the chromatographic sheets into pH 4.2 buffer and lightly blot each sheet between clean nonfluorescing, white blotters. Use separate sheets for the doxycycline standard solution, for each doxycycline sample solution, and for blanks without standard or sample application. Care must be taken so that the moist sheets do not become too dry; a period of 5 to 10 minutes between impregnating the paper and placing it in the chromatographic chamber is usually satisfactory. Evenly apply a 0.100-milliliter aliquot of a doxycycline solution to the origin line of a sheet as a 14-centimeter-long streak. Place the sheets in the chamber and develop them in a descending manner for 2 hours. The doxycycline band should move approximately 12.5 centimeters from the origin line. Remove the sheets from the chamber and air-dry for about 10 minutes.

(vi) *Processing the chromatogram*. Examine each sheet under 366-nanometer ultraviolet light. Outline the fluorescent bands with a pencil. The main marked area should be approximately 10 × 15 centimeters in size. Outline areas on the blank sheet approximately equal in size and in the same locations as those outlined on the standard sheet. Exposure of the sheets to ammonia or other alkaline vapors must be avoided. Cut the marked areas from the sheets and then cut them into approximately 2-centimeter squares. For each sheet, place the squares from each of the following areas into separate 125-milliliter Erlenmeyer flasks: The main doxycycline band of the sample, the main doxycycline band of the standard, all the other bands of the standard, the area of the blank sheet corresponding to the main band of the standard, the other area of the blank sheet corresponding to the other bands of the standard. The time between removing the sheets from the chamber and placing the squares into the Erlenmeyer

flasks should be minimal, since excessive drying of the paper can lead to erratic elutions.

(vii) *Elution.* To each flask add 50 milliliters of 0.05*N* methanolic hydrochloric acid and agitate on a reciprocating shaker for 1 hour. Decant the contents of each flask into another flask by pouring through a small funnel fitted with a glass wool plug.

(viii) *Doxycycline standard solution for direct measurement of absorbance.* Pipette a 0.100-milliliter aliquot of the doxycycline standard solution into each of three 125-milliliter Erlenmeyer flasks. Add 50 milliliters of 0.05*N* methanolic hydrochloric acid to each of these flasks.

(ix) *Absorbance measurement.* Using a suitable spectrophotometer and 0.05*N* methanolic hydrochloric acid as the reference solvent, determine the absorbance of each eluate and of each doxycycline standard solution at the absorption maximum at about 349 nanometers.

(x) *Calculation of percent doxycycline in samples.* Calculate as follows:

$$\text{Percent doxycycline} = \frac{(A_u - A_b)\,(W_s)}{(A_s - A_b)\,(W_u)} \times \text{Doxycycline content of the working standard}$$

where:

A_u=Absorbance of the eluate from the main doxycycline band of the sample sheet.

A_s=Absorbance of the eluate from the main doxycycline band on the standard sheet.

A_b=Absorbance of the eluate from the area of the blank sheet corresponding to the area of the doxycycline band of the standard sheet.

W_u=Weight in milligrams of sample.

W_s=Weight in milligrams of doxycycline working standard.

(xi) *Recovery of the doxycycline standard from the chromatogram. As follows:*

$$\text{Percent recovery} = \frac{As - Ab}{A_p} \times \frac{100}{F}$$

where:

A_p=Absorbance of the doxycycline standard solution described in paragraph (b)(5)(viii) of this section.

F=The fractional purity of doxycycline standard solution described in paragraph (b)(5)(xii) of this section.

If the recovery of the doxycycline standard from the chromatogram is less than 95 percent, repeat the chromatogram.

(xii) *Determination of the fractional purity of the doxycycline working standard.* Determine *F* by means of the following equation:

$$\mathrm{F} = 1 - \frac{Ac - Acb}{Ac - Acb + As - Ab}$$

where:

A_c=Absorbance of the eluate from sections of the standard chromatogram containing nondoxycycline 349 nanometers-absorbing contaminants.

A_{cb}=Absorbance of the eluates from the sections of the blank sheets corresponding to those sections of the nondoxycycline-absorbing contaminants of the standard sheets.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.20a Sterile doxycycline hyclate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, equality, and purity.* Sterile doxycycline hyclate is [4S - (4α,4aα,5α,5aα,6α 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide hydrochloride hemiethanolate hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(viii) It contains not less than 82 nor more than 90 percent doxycycline on an ''as is'' basis.

(ix) It gives a positive identity test for doxycycline hyclate.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 12 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(8) *Doxycycline content.* Proceed as directed in § 446.20(b)(5).

(9) *Identity.* Proceed as directed in § 436.211 of this subchapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.21 Doxycycline monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate is [4S - (4α,4aα,5α,5aα,6α,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, - 12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphtha - cenecarboxamide monohydrate. It is so purified and dried that:

(i) Its potency is not less than 880 micrograms nor more than 980 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.6 percent nor more than 4.6 percent.

(iv) Its pH in an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter is not less than 5.0 nor more than 6.5.

(v) It contains not less than 90 percent nor more than 98 percent doxycycline on an ''as is'' basis.

(vi) It gives a positive identity test for doxycycline monohydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content*. Proceed as directed in § 446.20(b)(5).

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.42 Meclocycline sulfosalicylate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Meclocycline sulfosalicylate is the sulfosalicylate salt of 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 620 micrograms of meclocycline per milligram on an ''as is'' basis.

(ii) Its moisture content is not more than 4.0 percent.

(iii) Its pH is in an aqueous suspension containing 10 milligrams per milliliter is not less than 2.5 and not more than 3.5.

(iv) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on potency, moisture, pH, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography method shall be conclusive.

(i) *High-pressure liquid chromatography*. Proceed as directed in § 436.329 of this chapter.

(ii) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 0.01*N* methanolic hydrochloric acid (solution 13) to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.06 microgram of meclocycline per milliliter (estimated).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams of meclocycline per milliliter.

(4) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[46 FR 3836, Jan. 16, 1981]

§ 446.50 Methacycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Methacycline hydrochloride is [4S - (4α,4aα,5α,5aα, - 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methylene - 1,11 - dioxo - 2 - naphthacenecar - boxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of methacycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) Its absorptivity at the absorption maximum of 345 nanometers relative to that of the methacycline working standard similarly treated is 92.4±4 percent.

(vi) It gives a positive result to the identity test for methacycline hydrochloride.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of methacycline per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 100 milliliters of 0.01*N* methanolic hydrochloric acid. Transfer a 10-milliliter aliquot to a 250-milliliter volumetric flask and dilute to volume with 0.01*N* methanolic hydrochloric acid. Using a suitable spectrphotometer and 0.01*N* methanolic hydrochloric acid as the blank, scan the absorption spectrum between the wavelengths of 250 and 400 nanometers. Determine the absorbance of each solution at the maxima, ca. 345 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

Percent relative absorptivity=(Absorbance of sample×weight in milligrams of standards×potency of standard in micrograms per milligram)/(Absorbance of standard×weight in milligrams of sample×10)

(6) *Identity.* The absorption spectrum between the wavelength of 250 and 400 nanometers, determined as directed in paragraph (b)(5) of this section, compares qualitatively with that of the methacycline standard.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.60 Minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride is [4S-(4α,4aα,5aα,12aα)]-4,7-bis (dimethylamino)-1,4,4a,5,5a,6,11, - 12a-octahydro-3,10,12, - 12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 890 micrograms per milligram and not more than 950 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams of minocycline per milliliter is not less than 3.5 and not more than 4.5.

(v) Its epi-minocycline content is not more than 1.2 percent.

(vi) It gives a positive identity test for minocycline hydrochloride.

(vii) It is crystalline.

(viii) Its residue on ignition is not more than 0.15 percent.

(ix) The absorptivity at 560 nanometers of an aqueous solution containing 10 milligrams of minocycline hydrochloride per milliliter is not more than 0.006.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, epiminocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Minocycline potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 4.6-millimeter × 3-centimeter guard column containing 10-micrometer diameter RP–8 Lichrosorb, a 4.6-millimeter × 15-centimeter analytical column packed with octyl silane chemically bonded to porous microsilica particles, 5 micrometers in diameter, a flow rate of 2.9 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.1 M Disodium ethylenediamine-tetraacetate (EDTA).* Accurately weigh 37.22 grams of disodium ethylenediaminetetraacetate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*b*) *0.2 M Ammonium oxalate.* Accurately weigh 28.42 grams of ammonium oxalate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*c*) *Mobile phase.* Mix 250 milliliters of dimethylformamide, 200 milliliters of 0.1*M* disodium ethylenediaminetetraacetate and 550 milliliters of 0.2*M* ammonium oxalate. (5:4:11). Allow the solution to cool to room temperature and then adjust the pH to 6.2 to 6.3 with 0.4*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chomatographic pumping system.

(ii) *Preparations of working standard, sample and resolution testing solutions*—(*a*) *Working standard solution.* Dissolve an accurately weighed portion of the minocycline hydrocholoride working standard with sufficient mobile phase (prepared as described in paragraph (b)(1)(i)(*c*) of this section) to obtain a solution containing 500 micrograms of minocycline activity per milliliter. Use this standard solution within 3 hours of preparation.

(*b*) *Sample solution.* Dissolve an accurately weighed sample in sufficient mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparations.

(iii) *System suitability requirements*—(*a*) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s)is satisfactory if it is not less than 0.9 and not more than 1.35.

(*b*) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in § 436.216(c)(2) calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;

n=number of theoretical plates; and

d_p=Average diameter of the particles in analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 50 for the minocycline peak.

(*c*) *Resolution.* Dissolve 50 milligrams of minocycline hydrochloride in 25 milliliters of deionized water. Pipet 5 milliliters of this solution into a 25-milliliter volumetric flask and heat on a steam bath for 60 minutes. Transfer the contents of the flask to a small beaker and evaporate to dryness. Dissolve the residue in mobile phase, transfer to a 25-milliliter volumetric flask, dilute to mark with mobile phase, mix, and filter through Whatman No. 1 filter

paper. Use this solution to determine the resolution factor. The resolution (R) between the peaks for minocycline and epi-minocycline is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(*e*) *Capacity factor (k′).* Calculate the capacity factor (*k′*) for minocycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of minocycline in minutes; and

t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;

L=Column length in centimeters;

0.75=Average total column porosity; and

F=Flow rate in milliliters per minute.

The capacity factor (*k′*) for minocycline is satisfactory if it is not less than 6.2 and not more than 11.5.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—Calculate the micrograms of minocycline per milligram of sample as follows:

$$\text{Micrograms of minocycline per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;

P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

C_u=Milligrams of minocycline sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

(5) *Epi-minocycline content.* Proceed as directed in paragraph (b)(1) of this section. Calculate the epi-minocycline content as follows:

$$\text{Percent Epi-minocycline} = \frac{(A_{epi}) \times 100}{(A_{total})}$$

where:

A_{epi}=Area of the epi-minocycline peak in the chromatogram of the sample; and

A_{total}=The sum of the areas of all the peaks eluting after the solvent front.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter.

(9) *Absorptivity.* Accurately weigh about 1 gram of sample into a 100-milliliter volumetric flask, dissolve, and dilute to mark with deionized water. Determine the absorbance of this solution on a suitable spectrophotometer at 560 nanometers (nm) using 5-centimeter cells with water in the reference cell. Calculate the absorptivity as follows:

$$\text{Absorptivity at 560 nm} = \frac{(A_{560})\ (100)}{(\text{grams of sample})\ (1{,}000)(5)}$$

[39 FR 19076, May 30, 1974, as amended at 43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32607, Aug. 26, 1988; 53 FR 39839, Oct. 12, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.65 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline is [4S-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimeth-

ylamino)-1,4,4a,5,5a,6,11,12a-octa - hydro- 3,5,6,10,12,12a-hexahydroxy-6-methyl- 1,11-dioxo-2-naphthacenecar - boxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 6 percent and not more than 9 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(v) When calculated on an anhydrous basis its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 100±4 percent.

(vi) It gives a positive result to an identity test for oxytetracycline.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.65a Sterile oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Sterile oxytetracycline is [4S - (4α,4aα,5α,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 6 percent and not more than 9 percent.

(vii) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytet- racycline working standard similarly treated, is 100±4 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milli- liter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of oxytetracycline per milliliter prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile, pyrogen-free distilled water.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter, preparing the sample by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(8) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.66 Oxytetracycline calcium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline calcium is [4S-(4α,4aα,5α,5aα,6β,12αβ)]-4-(dimethylamino)-1,4,4a,5,5a,6,11, 12a-octahydro-3,5,6,10,12,12a-hexa hydroxy-6-methyl-1,11-dioxo- 2-naphthacenecarboxamide calcium salt. Oxytetracycline is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is equivalent to not less than 865 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 8 percent and not more than 14 percent.

(iv) Its pH in an aqueous suspension containing 25 milligrams per milliliter is not less than 6.0 and not more than 8.0

(v) Its calcium content as the sulfated ash is not less than 3.85 percent and not more than 4.35 percent on an anhydrous basis.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, calcium content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous suspension containing 25 milligrams per milliliter.

(5) *Calcium content.* Proceed as directed in § 436.207(b) of this chapter, except from the weight of residue obtained calculate the calcium content as follows:

$$\text{Percent calcium} = \frac{\text{Weight of residue} \times 0.29435 \times 100 \times 100}{\text{Weight of sample (anhydrous basis)} \times (100 - m)}$$

where: m = Percent moisture in the sample.

(6) *Identity*. To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11157, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.67 Oxytetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline hydrochloride is [4S-(4α,4aα,5α,5aα, 6β,12aα,)] - 4 - (dimethylamino) - 1,4,4a,5, 5a,6, 11, 12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. Oxytetracycline is produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(v) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytetracycline standard similarly treated is 92.5±4.3 percent.

(vi) It gives a positive result to an identity test for oxytetracycline.

(vii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milli- liter (estimated).

(ii) *Chemical assay*. Proceed as directed in § 436.320 of this chapter.

(2) [Reserved]

(3) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard, using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *Identity*. To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11157, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.67a Sterile oxytetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile oxytetracycline hydrochloride is [4S - (4α,4aα,5α,5aα,6β,12α)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 92.5±4.3 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay*. Proceed as directed in § 436.320 of this chapter.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of oxytetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(7) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution

at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *Identity*. To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(10) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.75a Sterile rolitetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile rolitetracycline is [4S-(4α,4aα,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not more than 3.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7 and not more than 9, and such solution is substantially clear.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard similarly treated is 100±4.4 percent.

(x) It passes the identity test.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture*. Proceed as directed in § 436.201 of this subchapter.

(7) *pH*. Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this subchapter.

(9) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to separate 100-milliliter volumetric flasks. Add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each flask, and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where m=percent moisture in the sample.

(10) *Identity*. Place approximately 100 milligrams of the sample to be tested in a test tube, and 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, aminelike odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.76a Sterile rolitetracycline nitrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile rolitetracycline nitrate is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenecarboxamide mononitrate sesquihydrate. It is so purified and dried that:

(i) It contains not less than 765 micrograms of rolitetracycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard treated is 89.2±4.0 percent.

(x) It gives a positive result to the identity tests for rolitetracycline nitrate.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture*. Proceed as directed in § 436.201 of this subchapter.

(7) *pH*. Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this subchapter.

(9) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to representative 100-milliliter volumetric flasks. Add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where: *m*=percent moisture in the sample.

(10) *Identity*—(i) *Rolitetracycline*. Place approximately 100 milligrams of the sample to be used in a test tube, add 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, amine-like odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

(ii) *Nitrate identity*. Transfer approximately 1 gram of sample to a 250-milliliter beaker, add 100 milliliters of water, and acidify with 1 milliliter of acetic acid. Heat to boiling and, with constant stirring, add 10 milliliters of a 10-percent solution of nitron (1,4-diphenyl-3,5-endo-anilino-4,5-dihydro-1,2,4-triazole) $C_{20}H_{16}N_4$[7] in 1*N* acetic acid. Allow to cool. A heavy precipitate indicates the presence of nitrate.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11159, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.80 Tetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tetracycline is [4S -

[7] Nitron is available from J. T. Baker Laboratory Chemicals, North Phillipsburg, N.J. 08865.

(4α,4aα,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 975 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 13 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 3.0 and not more than 7.0.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 108.2±3.75 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It is crystalline.

(viii) It passes the identity test for tetracycline.

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, each package shall bear on its label or labeling the statement ''For use only in the manufacture of nonparenteral drugs.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetra- cycline content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 60 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample (as the anhydrous compound), accurately weighed, in 2.0 milliliters of 0.1*N* hydrochloric acid and dilute with distilled water to 250 milliliters. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \begin{matrix}\text{Potency of standard}\\ \text{in micrograms}\\ \text{per milligram}\end{matrix} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity.* Proceed as directed in § 436.308 of this chapter.

[43 FR 11159, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.81 Tetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 1.8 and not more than 2.8.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 100±4 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It is crystalline.

(viii) It passes the identity test for tetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample, accurately weighed, in approximately 150 milliliters of distilled water by mixing thoroughly. Dilute to 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0 milliliter aliquot of this solution to a 100-milliliter volumetric flask, add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \begin{matrix}\text{Potency of standard}\\ \text{in micrograms}\\ \text{per milligram}\end{matrix} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity.* Proceed as directed in § 436.308 of this chapter.

[43 FR 11159, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.81a Sterile tetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride is [4S - (4α,4aα,5aα,6β, 12aα)] - 4 - dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacene - carboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of tetracycline hydrochloride per milligram. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 1.8 and not more than 2.8.

(viii) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 100±4 percent.

(ix) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(x) It is crystalline.

(xi) It passes the identity test for tetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing.

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a stock solution containing 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated); also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a [so–]lution containing 5.0 milligrams of te–[tracycline] hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity*. Dissolve approximately 40 milligrams of the sample, accurately weighed, in approximately 150 milliliters of distilled water by mixing thoroughly. Dilute to 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \begin{matrix}\text{Potency of standard}\\ \text{in micrograms}\\ \text{per milligram}\end{matrix} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *4-Epianhydrotetracycline*. Proceed as directed in § 436.309 of this chapter.

(10) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(11) *Identity*. Proceed as directed in § 436.308 of this chapter.

[43 FR 11160, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 44 FR 31636, June 1, 1979; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.82 Tetracycline phosphate complex.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tetracycline phosphate complex is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide phosphate complex. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 9 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 2.0 and not more than 4.0.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 82.0±4.9 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It passes the identity test, showing a presence of phosphate, a content of not more than 0.2 percent chloride, and a content of not more than 1 percent tetracycline base.

(viii) It is crystalline.

(2) *Labeling*. In addition to the requirements of § 432.5 of this chapter, each such package shall bear on its label or labeling the statement ''For use only in the manufacture of nonparenteral drugs''.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, 4-epianhydro tetracycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 60 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of

the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a suspension containing 10 milligrams of the sample per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample, accurately weighed, in 2.0 milliliters of 0.1*N* HCl and dilute to 250 milliliters with distilled water. Transfer a 10.0 milliliter aliquot of this solution to a 100-milliliter volumetric flask, add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \begin{matrix}\text{Potency of standard}\\ \text{in micrograms}\\ \text{per milligram}\end{matrix} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(7) *Identity*—(i) *Presence of phosphate.* Prepare a filtrate as follows: Suspend 100 milligrams of the sample in 10 milliliters of distilled water and filter a small portion by gravity. Transfer 1.0 milliliter of the filtrate to a 100-milliliter glass-stoppered cylinder, add 10.0 milliliters of distilled water, 2.0 milliliters of ammonium molybdate test solution, 1.0 milliliter of stannous chloride test solution, and 10.0 milliliters of isobutyl alcohol-benzene mixture (1:1 ratio), all in the order named. Shake vigorously for 1 minute, allow the layers to separate, and examine the top organic layer. In the presence of phosphate, the top layer turns blue.

(ii) *Chloride content.* To 1.0 milliliter of the filtrate prepared as directed in the first sentence of paragraph (b)(7)(i) of this section, add 1 drop of silver nitrate test solution and 1 drop of nitric acid. Any turbidity produced is not greater than that obtained by similarly treating 1.0 milliliter of 0.057*N* hydrochloric acid.

(iii) *Determination of percent tetracycline base.* This test is used to determine the quantity of tetracycline present as base in mixtures with phosphate salts.

(*a*) *Reagents*—(*1*) 1,4-Dioxane.

(*2*) Purified dioxane: Pass the dioxane through a column of Amberlite IRA 400 (OH–) resin or equivalent.

(*3*) Perchloric acid, 0.01*N:* Dilute 0.84 milliliter of 70 percent perchloric acid to 1,000 milliliters with purified dioxane; standardize at least once every 2 days, as follows: Weigh accurately about 70 milligrams of diphenylguanidine, and dissolve in 50 milliliters of ethyl alcohol in a 250-milliliter flask. Add two drops of methyl red, and titrate with the perchloric acid solution until the yellow color changes to orange. Deduct the volume of the perchloric acid consumed by 50 milliliters of the ethyl alcohol, and calculate the normality. Each 2.113 milligrams of diphenylguanidine is equivalent to 1 milliliter of 0.01*N* perchloric acid.

(*4*) Methyl red indicator: Dissolve 100 milligrams of methyl red in 100 milliliters of methyl alcohol.

(*b*) *Procedure.* Place an accurately weighed 1-gram sample into a 50-milliliter Erlenmeyer flask, add 10.0 milliliters of purified dioxane and shake the mixture manually for about 2 minutes. Allow to settle, decant all the supernatant liquid into a 50-milliliter polyethylene centrifuge tube, cover with Parafilm (or equivalent), and centrifuge until clear (about 3 minutes).

Pipette 5.0 milliliters of the clear, supernatant solution into a 50-milliliter beaker, stir magnetically, and titrate with 0.01*N* perchloric acid, using methyl red as the indicator. The endpoint is the last color change to orange when a drop of titrant is added. Calculate the percent tetracycline base as follows:

$$\text{Percent tetracycline base} = \frac{\text{Milliliters of acid used} \times \text{Normality} \times 0.4445 \times 200}{\text{Weight of sample}}$$

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 11161, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart B—Oral Dosage Forms

§446.110 Chlortetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride capsules are composed of chlortetracycline hydrochloride and one or more suitable and harmless diluents, lubricants, and fillers. Each capsule contains 50, 100, or 250 milligrams of chlortetracycline hydrochloride. The potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. The loss on drying is not more than 1 percent. The chlortetracycline hydrochloride used conforms to the standards prescribed by §446.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Test and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.01*N* hydrochloric acid to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[43 FR 11162, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.115 Demeclocycline oral dosage forms.

§446.115a Demeclocycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives suspended in a suitable and harmless vehicle. Each milliliter contains demeclocycline equivalent to 15 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. The pH is not less than 4 and not more than 5.8. The demeclocycline used conforms to the standards prescribed by §446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Mix well. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 43 FR 50677, Oct. 31, 1978; 50 FR 19920, May 13, 1985]

§ 446.115b Demeclocycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline for oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains demeclocycline equivalent to 15 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. Its moisture content is not more than 5 percent. The demeclocycline used conforms to the standards prescribed by § 446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.116 Demeclocycline hydrochloride oral dosage forms.

§ 446.116a Demeclocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Demeclocycline hydrochloride tablets are composed of demeclocycline hydrochloride with one or more suitable and harmless diluents, lubricants, binders, and flavorings. Each tablet contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent. It shall disintegrate within 30 minutes. The demeclocycline hydrochloride used conforms to the standards prescribed by §446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter.

[39 FR 19076 , May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§446.116b [Reserved]

§446.116c Demeclocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride capsules are composed of demeclocycline hydrochloride, with one or more suitable and harmless diluents and lubricants, enclosed in a gelatin capsule. Each capsule contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent, except that if starch is used as a diluent the loss on drying is not more than 8 percent. The demeclocycline hydrochloride used conforms to the standards prescribed by §446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient

0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.120 Doxycycline hyclate oral dosage forms.

§ 446.120a Doxycycline hyclate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate capsules are composed of doxycycline hyclate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline hyclate equivalent to either 50, 100, or 300 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.0 percent. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline capsule contents and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final standard and sample solutions. The standard and sample must each produce a major, yellow fluorescent spot with the same R_f value, and the standard-sample mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974. Redesignated at 39 FR 41250, Nov. 26, 1974, and amended at 43 FR 11162, Mar. 17, 1978; 44 FR 20667, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 446.120b Doxycycline calcium oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline calcium oral suspension is prepared from doxycycline hyclate and contains one or more suitable and harmless buffer substances, preservatives, diluents, solvents, colorings, and flavorings. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of doxycycline that it is represented to contain. Its pH is not less than 6.5 and

not more than 8.0. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by §446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Transfer an appropriate aliquot of the suspension to a volumetric flask and dissolve with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration (containing not less than 150 micrograms of doxycycline per milliliter in acid). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

(3) *Identity.* Proceed as directed in §436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline calcium oral suspension and of the doxycycline working standard in methanol and further dilute each solution with methanol to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value, and the sample-standard mixed solution must show no separation of major spots.

[39 FR 41250, Nov. 11, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§446.120c Doxycycline hyclate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate tablets contain doxycycline hyclate with or without one or more disintegrants, lubricants, colorings, and coating substances. Each tablet contains doxycycline hyclate equivalent to 50 or 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. Its moisture content is not more than 5.0 percent. It passes the dissolution test. It passes the identity test. The doxycycline hyclate conforms to the standards prescribed by §446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, dissolution, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated).

Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter, except:

(i) In lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test; and

(ii) In lieu of paragraph (d) of that section, use the interpretation described in the United States Pharmacopeia XX dissolution test. The quantity, *Q* (the amount of doxycycline dissolved) is 55 percent at 60 minutes and 85 percent at 90 minutes.

(4) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the sample and standard solutions as follows: Grind tablet to a powder. Dissolve precise amount of the doxycycline tablet and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final standard and sample solutions. The standard and sample must each produce a major, yellow fluorescent spot with the same R_f value and the standard-sample mixed solution must show no separation of major spots.

[46 FR 7273, Jan. 23, 1981, as amended at 48 FR 23813, May 27, 1983; 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.120d Doxycycline hyclate pellet-filled capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate pellet-filled capsules contain pellets which are composed of doxycycline hyclate and suitable and harmless diluents, binders, and lubricants. Each capsule contains doxycycline hyclate equivalent to 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.0 percent. It passes the acid resistance test. It passes the dissolution test. The doxycycline hyclate conforms to the standards prescribed by § 446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, acid resistance, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Acid resistance.* Proceed as directed in § 436.543 of this chapter.

(4) *Dissolution.* Empty the contents of one pellet-filled capsule into the basket and proceed as directed in § 436.544 of this chapter. The quantity *Q* (the amount of doxycycline dissolved) is 85 percent at 30 minutes.

[50 FR 41679, Oct. 15, 1985, as amended at 55 FR 11584, Mar. 29, 1990]

§446.121 Doxycycline monohydrate oral dosage forms.

§446.121a Doxycycline monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate for oral suspension is doxycycline monohydrate with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. Its moisture content is not more than 3 percent. It passes the identity test for the presence of the doxycycline moiety. When prepared as directed in the labeling, each milliliter contains the equivalent of 5 milligrams of doxycycline and its pH is not less than 5.0 and not more than 6.5. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of doxycycline that it is represented to contain. The doxycycline monohydrate used conforms to the standards prescribed by §446.21(a)(1).

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, this drug shall be labeled ''doxycycline for oral suspension''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline monohydrate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in §436.202 of this chapter, using the undiluted sample.

(4) *Identity.* Proceed as directed in §436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline monohydrate for oral suspension and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 50 FR 19920, May 13, 1985. Redesignated at 55 FR 6637, Feb. 26, 1990]

§446.121b Doxycycline monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate capsules are composed of doxycycline monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline monohydrate equivalent to 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.5 percent. It passes the dissolution test. It passes the identity test. The doxycycline monohydrate used conforms to the standards prescribed by §446.21.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The doxycycline monohydrate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(B) The batch for potency, moisture, dissolution, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The doxycycline monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Doxycycline potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 4.6-millimeter X 3-centimeter guard column containing 5- to 10-micrometer diameter octyl silane chemically bonded to totally porous microsilica particles, a 3.9-millimeter X 30-centimeter analytical column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, a flow rate of 1.5 milliliters per minute, and a 10-microliter loop injector. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M sodium phosphate buffer.* Prepare a solution containing 13.8 grams of monobasic sodium phosphate per liter of distilled water.

(B) *Mobile phase.* Mix 450 milliliters of 0.1*M* monobasic sodium phosphate and 550 milliliters of methanol. Add 3 milliliters of *N,N*-dimethyl-*n*-octylamine. Adjust the pH to 8.0 with 5*N* sodium hydroxide. Filter the mobile phase through a suitable glass filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of the doxycycline hyclate working standard in sufficient 0.1*N* hydrochloric acid to obtain a known concentration of about 1,000 micrograms of doxycycline per milliliter. Further dilute with distilled water to a concentration of 40 micrograms of doxycycline activity per milliliter. Filter through a membrane filter of 0.5 micron or finer porosity.

(B) *Sample solution.* Remove, as completely as possible, the contents of a representative number of capsules. Mix the combined contents and transfer an accurately weighed portion of the powder, equivalent to about 100 milligrams of doxycycline, to a 100-milliliter volumetric flask. Add 20 milliliters of 0.1*N* hydrochloric acid and sonicate for 5 minutes. Dilute to mark with 0.1*N* hydrochloric acid. Further quantitatively dilute an aliquot of this solution with distilled water to a concentration of 40 micrograms of doxycycline activity per milliliter (estimated). Filter through a membrane filter of 0.5 micron or finer porosity. Content uniformity analyses may be obtained from sample solutions prepared as above except that the contents of one capsule are quantitatively transferred to the 100-milliliter volumetric flask.

(C) *Resolution test solution.* Dissolve 50 milligrams of doxycycline in 25 milliliters of distilled water. Pipet 5 milliliters of this solution into a 25-milliliter volumetric flask and heat on a steam bath for 60 minutes. Transfer the contents of the flask to a small beaker and evaporate to dryness. Dissolve the residue in distilled water, transfer to a 25-milliliter volumetric flask, dilute to mark with distilled water, mix, and filter through Whatman No. 1 filter paper. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to a point of a maximum peak height; and
b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 1.4 and not more than 2.0

(B) *Efficiency of the column*. From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;
n=number of theoretical plates; and
d_p=Average diameter of the particles in analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 37.5 for the doxycycline peak.

(C) The resolution (R) between peaks for doxycycline and epi-doxycycline is satisfactory if it is not less than 1.5.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′)*. Calculate the capacity factor (k') for doxycycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of doxycycline in minutes; and
t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;
L=Column length in centimeters;
0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (k') for doxycycline is satisfactory if it is not less than 1.5 and not more than 2.5. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system described. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations*. Calculate the doxycycline content as follows:

$$\text{Milligrams of doxycycline per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the doxycycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the doxycycline peak in the chromatogram of the working standard;
P_s=Doxycycline activity in the doxycycline working standard solution in micrograms per milliliter;
d=Dilution factor of the sample; and
n=Number of capsules in the sample assayed.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution*. Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of doxycycline dissolved) is 85 percent at 60 minutes.

(4) *Identity*. The high-pressure liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the doxycycline working standard.

[55 FR 6637, Feb. 26, 1990]

§ 446.150 Methacycline hydrochloride oral dosage forms.

§ 446.150a Methacycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Methacycline hydrochloride capsules are composed of methacycline hydrochloride and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains methacycline hydrochloride equivalent to either 70 milligrams of methacycline, 140 milligrams of methacycline, or 280 milligrams of methacycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of methacycline that

it is represented to contain. The moisture content is not more than 7.5 percent. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender jar containing sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 50 FR 19920, May 13, 1985]

§ 446.150b Methacycline hydrochloride oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride oral suspension contains methacycline hydrochloride and one or more suitable and harmless buffers, dispersants, diluents, colorings, flavorings, and preservatives. It contains methacycline hydrochloride equivalent to 14 milligrams of methacycline per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of methacycline that it is represented to contain. Its pH is not less than 6.5 nor more than 8.0. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required.

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask, and dilute to volume with sterile distilled water. Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.160 Minocycline hydrochloride oral dosage forms.

§ 446.160a Minocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride tablets are composed of minocycline hydrochloride and one or more suitable

and harmless diluents, binders, lubricants, coloring, and coating substances. Each tablet contains minocycline hydrochloride equivalent to 100 milligrams of minocycline. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The tablets disintegrate within 30 minutes. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Grind a representative number of tablets in a mortar and pestle. Wash the ground tablets into a volumetric flask containing mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) and shake to dissolve. Dilute with mobile phase to give a stock solution of convenient concentration. Filter the stock solution. Further dilute using mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations.* Calculate the minocycline content as follows:

$$\text{Milligrams of minocycline per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times 5}$$

where:

A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;

P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§ 446.160b Minocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride capsules are composed of minocycline hydrochloride and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains minocycline hydrochloride equivalent to 50 or 100 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content,

identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution*. Open a representative number of capsules and empty the contents into a volumetric flask containing mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) and shake to dissolve. Dilute with mobile phase to give a stock solution of convenient concentration. Filter the stock solution. Remove an aliquot of the stock solution and further dilute with mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations*. Calculate the minocycline content as follows:

$$\text{Milligrams of minocycline per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of capsules in the sample assayed.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§ 446.160c Minocycline hydrochloride oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Minocycline hydrochloride oral suspension is minocycline hydrochloride with one or more suitable flavorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains minocycline hydrochloride equivalent to 10 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of minocycline that it is represented to contain. Its pH is not less than 7.0 and not more than 9.0. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution*. Transfer an accurately measured 5-milliliter portion of the well-shaken suspension to a 100-milliliter volumetric flask. Dilute to mark with mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) and mix well. Filter this solution and use within 3 hours of its preparation.

(ii) *Calculations*. Calculate the minocycline content as follows:

$$\text{Milligrams of minocycline per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times 5}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *pH.* Proceed as directed in §436.202 of this subchapter, using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§446.165 Oxytetracycline oral dosage forms.

§446.165a Oxytetracycline tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline tablets are tablets composed of oxytetracycline and one or more suitable and harmless, diluents, binders, lubricants, colorings, and coating substances. The potency of each tablet is 250 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. The moisture content is not more than 7.5 percent. They shall disintegrate within 1 hour. The oxytetracycline used conforms to the standards prescribed by §446.65(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the method described in paragraph (e)(1) of that section.

[43 FR 11163, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§§446.165b—446.165c [Reserved]

§446.165d Oxytetracycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline for oral suspension is oxytetracycline with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When prepared as directed in the labeling, each milliliter contains 50 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its loss on drying is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The oxytetracycline used conforms to the standards prescribed by §446.65(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Mix well. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

[43 FR 11164, Mar. 17, 1978, as amended at 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.166 Oxytetracycline calcium oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline calcium oral suspension contains oxytetracycline calcium with one or more suitable and harmless buffer substances, suspending and stabilizing agents, flavorings, colorings, solvents, and preservatives suspended in a suitable and harmless vehicle. It may contain *N*-acetyl glucosamine. Each milliliter contains a quantity of oxytetracycline calcium equivalent to 25 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its pH is not less than 5.0 and not more than 8.0. The oxytetracycline calcium used conforms to the standards prescribed by § 446.66(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline calcium used in making the batch for potency, moisture, pH, calcium content, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The oxytetracycline calcium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the sample to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[43 FR 11164, Mar. 17, 1978 as amended at 43 FR 50677, Oct. 31, 1978; 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.167 Oxytetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride capsules are gelatin capsules

containing oxytetracycline hydrochloride with or without one or more suitable and harmless buffers, preservatives, diluents, binders, and lubricants. They may contain glucosamine hydrochloride. Each capsule contains 50 milligrams, 100 milligrams, 125 milligrams, or 250 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. The loss on drying is not more than 5.0 percent. It passes the dissolution test. The oxytetracycline hydrochloride used conforms to the standards prescribed by §446.67(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, and dissolution.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *Dissolution.* Proceed as directed in §436.215 of this chapter, except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of oxytetracycline dissolved) is 60 percent within 30 minutes and 85 percent within 60 minutes.

[43 FR 11164, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 3, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§446.180 Tetracycline oral dosage forms.

§§446.180a—446.180b [Reserved]

§446.180c Tetracycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline oral suspension is composed of tetracycline with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives, suspended in a suitable and harmless vehicle. Each milliliter contains tetracycline equivalent to 25 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its pH is not less than 3.5 and not more than 6.0. Its 4-epianhydrotetracycline content is not more than 5.0 percent. The tetracycline used conforms to the standards prescribed by §446.80(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline used in making the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

(3) *4-Epianhydrotetracycline*. Proceed as directed in § 436.309(b) of this chapter.

[43 FR 11164, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 45 FR 16472, 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.181 Tetracycline hydrochloride oral dosage forms.

§§ 446.181a—446.181c [Reserved]

§ 446.181d Tetracycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Tetracycline hydrochloride tablets contain tetracycline hydrochloride with or without one or more buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 250 milligrams or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 3.0 percent. It passes the dissolution test. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, dissolution, and 4-epianhydrotetracycline content.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(3) *Dissolution*. Proceed as directed in § 436.215 of this chapter, except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of tetracycline hydrochloride dissolved) is 60 percent within 30 minutes and 85 percent within 60 minutes.

(4) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11165, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 8, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§ 446.181e Tetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride capsules are composed of tetracycline hydrochloride with or without one or more suitable and harmless buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings enclosed in a gelatin capsule. Each capsule contains 50, 100, 125, 250, or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 4 percent. Its 4-epianhydrotetracycline content is not more than 3.0 percent. It passes the dissolution test. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, 4-epianhydrotetracycline content, and dissolution.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(4) *Dissolution.* Proceed as directed in § 436.215 of this chapter except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of tetracycline hydrochloride dissolved), except for the 500-milligram capsule, is 60 percent within 30 minutes and 85 percent within 60 minutes. For the 500-milligram capsule, the quantity *Q* is 50 percent within 30 minutes, 70 percent within 60 minutes, and 85 percent within 90 minutes.

[43 FR 11166, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 8, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§ 446.182 Tetracycline phosphate complex capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex capsules contain tetracycline phosphate complex with or without one or more buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings enclosed in a gelatin capsule. Each capsule contains tetracycline phosphate complex equivalent to 50, 100, 125, 250, or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 9.0 percent. Its 4-

epianhydrotetracycline content is not more than 3.0 percent. The tetracycline phosphate complex used conforms to the standards prescribed by § 446.82 (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification, samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline phosphate complex used in making the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetracycline content, identity, and crystallinity.

(b) The batch for potency, loss on drying, and 4-epianhydrotetracycline content.

(ii) Samples required:

(a) The tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11166, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 446.220 Doxycycline hyclate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate for injection is a dry mixture of doxycycline hyclate and a buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 2.0 percent. Its pH when reconstituted as directed in the labeling is not less than 1.8 and not more than 3.3. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 0.1*N* hydrochloric

acid to give a stock solution of convenient concentration (containing not less than 150 micrograms of doxycycline in acid). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(a) of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in the labeling.

(8) *Identity.* Proceed as directed in § 436.308 of this subchapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline hyclate for injection and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11166, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.260 Sterile minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile minocycline hydrochloride is a lyophilized powder of minocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of minocycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substance. Its moisture content is not more than 3.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.5. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, moisture, and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, withdraw an accurately measured representation portion from each container. Dilute the sample thus obtained with sufficient mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) to give a stock solution of convenient concentration. Filter the stock solution. Further dilute an aliquot of this stock solution with mobile phase to obtain a solution containing 500 micrograms of

minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations—(a)* Calculate the minocycline content of the single-dose vial as follows:

$$\text{Milligrams of minocycline per single-dose vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(*b*) Calculate the minocycline content of the multiple-dose vial as follows:

$$\text{Milligrams of minocycline per multiple-dose vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample.

n = Volume of sample solution assayed.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11166, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.265 Oxytetracycline injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline injection is a solution of oxytetracycline with or without one or more suitable and harmless buffer substances, anesthetics, preservatives, antioxidants, complexing agents, and solvents. Each milliliter contains 50 milligrams or 125 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its pH is not less than 8.0 and not more than 9.0. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1), except sterility, pyrogens, and depressor substances.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, and pH.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for

assay as follows: Transfer an accurately measured representative quantity of the sample to an appropriate-sized volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of oxytetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[43 FR 11166, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.267 Oxytetracycline hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline hydrochloride for injection is a dry mixture of oxytetracycline hydrochloride and a suitable buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 3.0 percent. Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 1.8 and not more than 2.8. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1), except sterility, pyrogens, and depressor substances.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, promptly remove all the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from the container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in 436.32(b) of this chapter, using a solution containing 5.0 milligrams per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this chapter,

using the diluent recommended by the manufacturer in the labeling for the drug.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 25 milligrams per milliliter.

[43 FR 11167, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.275 Rolitetracycline injectable dosage forms.

§ 446.275a Rolitetracycline for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline for intravenous use is a dry mixture of rolitetracycline and one or more suitable buffer substances. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by § 446.75a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11167, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.275b Rolitetracycline for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline for intramuscular use is a dry mixture of rolitetracycline and one or more suitable buffer substances and anesthetic agents. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is

represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by §446.75a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline used in making the batch for potency, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in §436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11167, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.276 Rolitetracycline nitrate injectable dosage forms.

§446.276a Rolitetracycline nitrate for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline nitrate for intravenous use is a dry mixture of rolitetracycline nitrate and one or more suitable buffer substances. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by §446.76a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is [repre-]sented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying*. Proceed as directed in § 436.200(b) of this subchapter.

(7) *pH*. Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11168, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.276b Rolitetracycline nitrate for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rolitetracycline nitrate for intramuscular use is a dry mixture of rolitetracycline nitrate, one or more suitable buffer substances, and lidocaine hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by § 446.76a(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in §436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11168, Mar. 30, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.281 Tetracycline hydrochloride injectable dosage forms.

§446.281a Sterile tetracycline hydrochloride.

The requirements for certification and the tests and methods of assay for sterile tetracycline hydrochloride packaged for dispensing are described in §446.81a.

§446.281c Tetracycline hydrochloride for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for intramuscular use is a dry mixture of tetracycline hydrochloride, magnesium chloride, or magnesium ascorbate and one or more suitable buffer substances, with or without one or more suitable preservatives and anesthetic agents, and with or without one or more suitable solubilizers and stabilizers. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by §446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, depressor substances, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[44 FR 31636, June 1, 1979, as amended at 46 FR 60568, Dec. 11, 1981; 47 FR 13326, Mar. 30, 1982; 50 FR 19920, May 13, 1985]

§ 446.281d Tetracycline hydrochloride for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for intravenous use is a dry mixture of tetracycline hydrochloride with one or more suitable and harmless stabilizing agents. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, cystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum for 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawal contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[44 FR 31636, June 1, 1979, as amended at 46 FR 60568, Dec. 11, 1981; 47 FR 13326, Mar. 30, 1982; 50 FR 19920, May 13, 1985]

§ 446.282 Tetracycline phosphate complex for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex for injection is a dry mixture of tetracycline phosphate complex, magnesium chloride or magnesium ascorbate and one or more suitable buffer substances, with or without one

or more suitable preservatives and anesthetic agents, and with or without one or more suitable solubilizers and stabilizers. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline phosphate complex conforms to the standards prescribed by § 446.82(a)(1), and it contains no depressor substance.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline phosphate complex used in making the batch for potency, moisture, pH, depressor substances, absorptivity, 4-epianhydrotetracycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately 300 milligrams, and one package containing approximately 1 gram.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams of sample.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *Depressor substances in the tetracycline phosphate complex used in making the batch.* Proceed as directed in § 436.35 of this chapter. Prepare the test solution by dissolving 40 milligrams of sample in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with sterile distilled water (diluent 3) to the prescribed concentration.

(7) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11168, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

Subpart D—Ophthalmic Dosage Forms

§ 446.310 Chlortetracycline hydrochloride ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride ophthalmic ointment contains chlortetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 10 milligrams of chlortetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes

the test for metal particles. The chlortetracycline hydrochloride used conforms to the standards prescribed by § 446.10a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(b) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[43 FR 11169, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.367 Oxytetracycline hydrochloride ophthalmic dosage forms.

§ 446.367c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic suspension is oxytetracycline hydrochloride and hydrocortisone acetate in a suitable and harmless oil base containing aluminum tristearate. Each milliliter contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and 15 milligrams of hydrocortisone acetate. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of five immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured, representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of the sample in lieu of 1.0 milliliter.

(3) *Moisture*. Proceed as directed in §436.201 of this chapter.

[43 FR 11169, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.367e Oxytetracycline hydrochloride-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline hydrochloride-polymyxin B sulfate ophthalmic ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 1 percent. It passes the test for metal particles. The oxytetracycline hydrochloride used conforms to the standards prescribed by §446.67a (a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1) of this chapter.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch:

(1) For all tests except sterility: A minimum of 16 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the ointment and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference

concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washings until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[43 FR 11170, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.381 Tetracycline hydrochloride ophthalmic dosage forms.

§ 446.381a Tetracycline hydrochloride ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ophthalmic ointment contains tetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 10 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of 3 more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot with sterile distilled water to the reference concentration of 0.24 micrograms of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[43 FR 11170, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.381b Tetracycline hydrochloride ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ophthalmic suspension contains tetracycline hydrochloride in a suitable and harmless oily base. Each milliliter contains 10 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into a high-speed glass blender jar with 1 milliliter polysorbate 80 and sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less that 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11171, Mar. 17, 1978, as amended at 46 FR 46313, Sept. 18, 1981; 50 FR 19920, May 13, 1985]

Subpart E—Otic Dosage Forms

§ 446.467 Oxytetracycline hydrochloride-polymyxin B sulfate otic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate otic ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram of ointment contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline hydrochloride content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1.0 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washings until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11171, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart F—Dermatologic Dosage Forms

§ 446.510 Chlortetracycline hydrochloride ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Chlortetracycline hydrochloride ointment contains chlortetracycline hydrochloride and one or more suitable and harmless preservatives in a suitable and harmless ointment base. Each gram contains 30 milligrams of chlortetracycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. Its moisture content is not more than 0.5 percent. The chlortetracycline hydrochloride used conforms to the standards prescribed by § 446.10(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 60 milligrams.

(*b*) The batch: A minimum of five immediate containers:

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11172, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.542 Meclocycline sulfosalicylate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Meclocycline sulfosalicylate cream contains meclocycline sulfosalicylate in a suitable and harmless cream base. Each gram contains meclocycline sulfosalicylate equivalent to 10 milligrams of meclocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of meclocycline that it is represented to contain. The meclocycline sulfosalicylate used conforms to the standards prescribed by § 446.42(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The meclocycline sulfosalicylate used in making the batch for potency, moisture, pH, and crystallinity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The meclocycline sulfosalicylate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency*. Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography method shall be conclusive.

(1) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient 0.01*N* methanolic hydrochloric acid (solution 13) to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.06 microgram of meclocycline per milliliter (estimated).

(2) *High-pressure liquid chromatography*. Proceed as directed in

§ 436.329 of this chapter, except, prepare the working standard and sample solution and calculate the meclocycline content as follows:

(i) *Preparation of standard solution.* Accurately weigh an amount of working standard equivalent to approximately 25 milligrams of meclocycline into a 50-milliter volumetric flask. Dissolve and dilute to volume with methanol and mix. Transfer exactly 2.0 milliliters of this solution to a 100-milliliter volumetric flask, dilute to volume with mobile phase, and mix.

(ii) *Preparation of sample solution.* Accurately weigh approximately 0.4 to 0.7 gram of sample into a 50-milliliter glass-stoppered centrifuge tube. Add 20 milliliters of methanol and 20 milliliters of 0.025*N* sulfuric acid. Disperse the sample thoroughly by a combination of ultrasonic/vortexing and shaking by hand. Shake for 15 minutes on a wrist action shaker. Quantitatively transfer the contents of the centrifuge tube into a 50-milliliter volumetric flask. Rinse the centrifuge tube with two 5-milliliter portions of methanol and add to the flask. Dilute to volume with methanol and mix. Transfer a portion of the content of the volumetric flask into an appropriate-sized centrifuge tube. Centrifuge for 5 minutes at 2,000 revolutions per minute. Transfer 5.0 milliliters of this solution into a 50-milliliter volumetric flask and dilute to volume with mobile phase and mix. Filter this solution through a 0.5 micrometer filter. Inject the filtrate onto the column as described in § 436.329(e) of this chapter.

(iii) *Calculations.* Calculate the meclocycline content as follows:

$$\text{Meclocycline content of cream in percent} = \frac{A \times 2 \times \text{milligrams of working standard} \times \text{Potency of working standard in micrograms per milligram}}{B \times 100 \times \text{milligrams of sample}}$$

where:

A=Area or peak height of the sample peak (at a retention time equal to that observed for the standard);

B=Area or peak height of the standard peak.

[46 FR 3837, Jan. 16, 1981; 46 FR 21361, Apr. 10, 1981, as amended at 47 FR 22515, May 25, 1982; 50 FR 1504, Jan. 11, 1985]

§ 446.567 Oxytetracycline hydrochloride dermatologic dosage forms.

§ 446.567a [Reserved]

§ 446.567b Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains oxytetracycline hydrochloride equivalent to 30 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or within the package: Adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the ointment and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washing until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11172, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.567c Oxytetracycline hydrochloride-polymyxin B sulfate topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical powder is oxytetracycline hydrochloride and polymyxin B sulfate with a suitable filler. Each gram contains 30 milligrams of oxytetracycline and 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. The loss on drying is not more than 2.0 percent. The oxytetracycline hydrochloride used conforms to the

standards prescribed by § 446.67. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions for lay use of the drug.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(b) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin content, and loss on drying.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh 1 gram of the powder and place into a 50-milliliter centrifuge tube. Add 15 milliliters of acetone and 1 drop of concentrated hydrochloric acid and stir well. Add 20 milliliters of acetone and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant liquid and repeat the acetone-acid extraction once more. Dissolve and dilute the residue with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[43 FR 11173, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.581 Tetracycline hydrochloride dermatologic dosage forms.

§§ 446.581a—446.581b [Reserved]

§ 446.581c Tetracycline hydrochloride for topical solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for topical solution is a dry mixture of tetracycline hydrochloride, 4-epitetracycline hydrochloride, and sodium bisulfite packaged in combination with a suitable and harmless aqueous vehicle. When reconstituted as directed in the labeling, each milliliter contains 2.2 miligrams of tetracycline hydrochloride. The tetracycline hydrochloride content of the reconstituted solution is satisfactory if it contains not less than 90 percent and not more

than 130 percent of the number of milligrams of tetracycline hydrochloride per milliliter that it is represented to contain. The 4-epitetracycline hydrochloride content is satisfactory if it contains not less than 135 percent and not more than 165 percent of the amount of tetracycline hydrochloride in the reconstituted solution at the time of reconstitution. The loss on drying of the dry mixture is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.9 and not more than 3.5. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a, except sterility, pyrogens, and histamine. The 4-epitetracycline hydrochloride used conforms to the following standards: It gives a positive identity test for 4-epitetracycline hydrochloride; its 4-epitetracycline content is not less than 70 percent; its total anhydrotetracycline and 4-epianhydrotetracycline content is not more than 2.0 percent; its loss on drying is not more than 6.0 percent; its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 4.0.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, and crystallinity.

(*b*) The 4-epitetracycline hydrochloride used in making the batch for 4-epitetracycline content and identity, total anhydrotetracycline and 4-epianhydrotetracycline content, loss on drying, and pH.

(*c*) The batch for tetracycline hydrochloride content, 4-epitetracycline hydrochloride content, loss on drying, and pH.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay of the tetracycline hydrochloride for topical solution*—(1) *Tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.* Proceed as directed in § 436.340 of this chapter.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, except use the contents of one immediate container.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when reconstituted as directed in the labeling.

(c) *Tests and methods of assay of the 4-epitetracycline hydrochloride used in making the batch*—(1) *4-epitetracycline content and identity.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of § 446.581c(b)(1)(iv) prepare the sample by weighing accurately 20 milligrams ±5 milligrams of 4-epitetracycline hydrochloride bulk powder and transfer to a 25-milliliter volumetric flask. Dissolve with 1.0 milliliter of methyl alcohol and dilute to volume with the buffer solution. Pipet a 2.0-milliliter aliquot to a 10-milliliter volumetric flask and dilute to volume with the buffer solution. Place the column in a suitable support. Place a 100-milliliter graduate under the column. Open the column stopcock, pipet 2.0 milliliters of solution from the 10-milliliter volumetric flask onto the column packing and allow the sample to permeate the column packing. Place a solvent reservoir containing 20 milliliters of benzene on top of the column and begin to collect the eluate (at flow rate of approximately 1 milliliter per minute). When the benzene level reaches the top of the column packing, replace the empty solvent reservoir with a second solvent reservoir containing 60 milliliters of chloroform and continue elution. When the chloroform level reaches the top of the column packing, replace second empty solvent reservoir with a third solvent reservoir containing 50-milliliters of the *n*-butanol:chloroform mixture and replace the 100-milliliter graduate with a 10-milliliter graduate. Collect 8.0 milliliters of eluate. Replace the 10-milliliter graduate with a 50-milliliter low-actinic volumetric flask and continue collecting the eluate containing the 4-

epitetracycline fraction until the column runs dry. Determine the absorbance of the 4-epitetracycline eluate as described in paragraph (b)(1)(v) of this section.

Calculate the 4-epitetracycline hydrochloride content of the 4-epitetracycline hydrochloride bulk powder as follows:

$$\text{Percent by weight 4-epitetracycline content} = \frac{A \times 25 \times 10 \times 50 \times 100}{a \times W \times 2 \times 2}$$

where:

A=Absorbance at 366 nanometers of the low-actinic 50-milliliter volumetric flask.

a=Previously established mean absorptivity of the tetracycline hydrochloride working standard eluates in liters/gram/centimeter with the calculation corrected for potency.

W=Weight of 4-epitetracycline hydrochloride bulk powder in milligrams.

The identity of the 4-epitetracycline hydrochloride is confirmed if the absorbance of the sample after column elution is such that the 4-epitetracycline hydrochloride content is greater than 70 percent by weight.

(2) *Total anhydrotetracycline and 4-epianhydrotetracycline content.* Proceed as directed in § 436.309 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

[42 FR 59066, Nov. 15, 1977; 43 FR 3705, Jan. 27, 1978, as amended at 48 FR 51291, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.581d Tetracycline hydrochloride ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ointment contains tetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 30 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its moisture content is not more than 1 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1), except 4-epianhydrotetracycline content.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or inserted within the package: Adequate directions under which the layperson can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than

150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11174, Mar. 17, 1978 as amended at 44 FR 30333, May 25, 1979. Redesignated at 45 FR 16472, Mar. 14, 1980, and amended at 50 FR 19920, May 13, 1985]

Subpart G—Vaginal Dosage Forms

§ 446.667 Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets are tablets composed of oxytetracycline hydrochloride and polymyxin B sulfate with one or more suitable diluents, binders, lubricants, and preservatives. Each tablet contains oxytetracycline hydrochloride equivalent to 100 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 100,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. The loss on drying is not more than 3.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and loss on drying.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Grind a representative number of tablets into a fine powder and place this powder, accurately weighed, into a filter funnel with a solvent-resistant membrane filter of 1.0 micrometer porosity. Wash the powder with five 20-milliliter portions of acetone or until the yellow color has disappeared. Remove the filter and soak in 400 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and blend. Quantitatively transfer to a 500-milliliter volumetric flask and adjust to volume with solution 6. Further dilute an aliquot with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[43 FR 11174, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart H—Rectal Dosage Forms [Reserved]

Subpart I [Reserved]

Subpart J—Certain Other Dosage Forms [Reserved]

PART 448—PEPTIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
448.10 Bacitracin.
448.10a Sterile bacitracin.
448.13 Bacitracin zinc.
448.13a Sterile bacitracin zinc.
448.15a Sterile capreomycin sulfate.
448.20a Sterile colistimethate sodium.
448.21 Colistin sulfate.
448.23 Cyclosporine.
448.25 Gramicidin.
448.30 Polymyxin B sulfate.
448.30a Sterile polymyxin B sulfate.
448.75 Tyrothricin.

Subpart B—Oral Dosage Forms

448.121 Colistin sulfate for oral suspension.
448.123 Cyclosporine oral dosage forms.
448.123a Cyclosporine oral solution.
448.123b Cyclosporine capsules.

Subpart C—Injectable Dosage Forms

448.210 Sterile bacitracin.
448.215 Sterile capreomycin sulfate.
448.220 Colistimethate sodium injectable dosage forms.
448.220a Sterile colistimethate sodium.
448.223 Cyclosporine for infusion.
448.230 Sterile polymyxin B sulfate.

Subpart D—Ophthalmic Dosage Forms

448.310 Bacitracin ophthalmic dosage forms.
448.310a [Reserved]
448.310b Bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment.
448.310c Bacitracin ophthalmic ointment.
448.313 Bacitracin zinc ophthalmic dosage forms.
448.313a Bacitracin zinc-polymyxin B sulfate ophthalmic ointment.
448.313b Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ophthalmic ointment; bacitracin zinc-neomycin sulfate-polymyxin B sulfate hydrocortisone ophthalmic ointment.
448.321 Colistin sulfate for ophthalmic solution.
448.330 Polymyxin B sulfate-trimethoprim hemisulfate ophthalmic solution.

Subpart E—Otic Dosage Forms

448.421 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.
448.430 Polymyxin B sulfate-hydrocortisone otic solution.

Subpart F—Dermatologic Dosage Forms

448.510 Bacitracin dermatologic dosage forms.
448.510a Bacitracin ointment.
448.510b—448.510c [Reserved]
448.510d Bacitracin-neomycin sulfate ointment.
448.510e Bacitracin–neomycin sulfate-polymyxin B sulfate ointment.
448.510f Bacitracin-polymyxin B sulfate topical aerosol.
448.513 Bacitracin zinc dermatologic dosage forms.
448.513a Bacitracin zinc-polymyxin B sulfate ointment.
448.513b Bacitracin zinc-neomycin sulfate ointment.
448.513c Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ointment; bacitracin zinc-neomycin sulfate-polymyxin B sulfate hydrocortisone ointment.
448.513d Bacitracin zinc-polymyxin B sulfate topical powder.
448.513e Bacitracin zinc-polymyxin B sulfate topical aerosol.
448.513f Bacitracin zinc ointment.

Subpart G—Vaginal Dosage Forms [Reserved]

Subparts H–I [Reserved]

Subpart J—Certain Other Dosage Forms

448.910 Bacitracin for prescription compounding.
448.913 Bacitracin zinc for prescription compounding.
448.930 Polymyxin B sulfate in certain other dosage forms.
448.930a Polymyxin B sulfate for prescription compounding.
448.930b Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19115, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§448.10 Bacitracin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin is a white to brown, neutral water-soluble polypeptide. It is so purified and dried that:

(i) Its potency is not less than 40 units of bacitracin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5 percent.

(iv) Its pH in an aqueous solution containing 10,000 units per milliliter is not less than 5.5 and not more than 7.5.

(v) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 1.0 gram.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin zinc in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Remove an aliquot of the stock solution, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10,000 units per milliliter.

(5) *Identity.* Proceed as directed in §436.319 of this chapter.

[42 FR 27229, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§448.10a Sterile bacitracin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin is a white to brown, neutral water-soluble polypeptide. It is so purified and dried that:

(i) Its potency is not less than 50 units per milligram. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units of bacitracin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 5 percent.

(vi) Its pH in an aqueous solution containing 10,000 units per milliliter is not less than 5.5 and not more than 7.5.

(vii) Its residue on ignition is not more than 3.0 percent.

(viii) It passes the identity test.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, identity, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 1 gram.

(*2*) For sterility testing: 1 package, containing approximately 12 grams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests ands methods of assay*—(1) *Potency.* Proceed as directed for bacitracin zinc in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed

sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 1 to obtain a stock solution of convenient concentration. Remove an aliquot of the stock solution, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 300 units of bacitracin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10,000 units per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(8) *Identity.* Proceed as directed in § 436.319 of this chapter.

(9) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[42 FR 27229, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.13 Bacitracin zinc.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc is the zinc salt of a kind of bacitracin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 40 units per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5 percent.

(iv) Its pH is not less than 6.0 and not more than 7.5.

(v) Its zinc content is not more than 10 percent by weight on an anhydrous basis.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, each package shall bear on the outside wrapper or container and the immediate container the statement ''For use only in the manufacture of nonparenteral drugs''.

(3) *Requests for certification; samples.* In addition to complying with the requriements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, zinc content,and identity.

(ii) Samples required: 10 packages, each containing approximately 1.0 gram.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute an aliquot with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

NOTE: The final sample solution must contain the same amount of hydrochloric acid as the reference concentration of the working standard.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated solution (approximately 100 milligrams of the sample per milliliter).

(5) *Zinc content.* Proceed as directed in § 436.312 of this chapter.

(6) *Identity.* Proceed as directed in § 436.319 of this chapter.

[42 FR 27229, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.13a Sterile bacitracin zinc.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Sterile bacitracin zinc is the zinc salt of a kind of bacitracin or a mixture of two or more such salts. It is so purified and dried that:

(i) It contains not less than 40 units of bacitracin per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) Its pH is not less than 6.0 and not more than 7.5.

(vi) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(vii) It passes the identity test.

(2) *Labeling.* In addition to the labeling requirements of §432.5 of this chapter, each package shall bear on the outside wrapper or container and the immediate container the statement ''For use in the manufacture of topical drugs only''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, loss on drying, pH, zinc content, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: Six packages, each containing approximately 1.0 gram.

(*b*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin in §436.105 of this chapter, except add to each standard response line concentration sufficient 0.01*N* hydrochloric acid to yield the same ratio of 0.01*N* hydrochloric acid to 1 percent potassium phosphate buffer, pH 6.0 (solution 1) as present in the sample solution diluted to the reference concentration. Prepare the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid F in lieu of diluting fluid A.

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using a saturated solution (approximately 100 milligrams of the sample per milliliter).

(6) *Zinc content.* Proceed as directed in §436.312 of this chapter.

(7) *Identity.* Proceed as directed in §436.319 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 40 FR 15088, Apr. 4, 1975; 40 FR 19194, May 2, 1975; 42 FR 27230, May 27, 1977; 50 FR 19920, May 13, 1985]

§448.15a Sterile capreomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile capreomycin sulfate is the amorphous sulfate salt of capreomycin. It is a white or essentially white powder. Capreomycin has been separated chromatographically into components designated capreomycins Ia, Ib, IIa, and IIb. Each component has been partially characterized according to its type and amino acid content. Capreomycin Ia contains serine and no alanine. Capreomycin Ib contains alanine and no serine. Capreomycin I is a mixture of capreomycins Ia and Ib. It is so purified and dried that:

(i) Its potency is not less than 700 micrograms and not more than 1,050 micrograms of capreomycin per milligram on an ''as is'' basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of capreomycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) It contains no depressor substance.

(vi) Its loss on drying is not more than 10 percent.

(vii) Its pH in an aqueous solution containing 30 milligrams per milliliter (or if packaged for dispensing, after reconstitution as directed in the labeling) is not less than 4.5 and not more than 7.5.

(viii) Its capreomycin I content is not less than 90 percent of the total capreomycins.

(ix) Its residue on ignition is not more than 3 percent.

(x) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, capreomycin I content, residue on ignition, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 100 micrograms of capreomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 30 milligrams per milliliter; however, if it is packaged for dispensing, use the solution obtained after reconstituting the drug as directed in the labeling.

(8) *Capreomycin I content*—(i) *Equipment*—(*a*) *Sheet* (*chromatographic*). Whatman No. 1 filter paper for chromatography 20 × 50 centimeters.

(*b*) *Chamber* (*chromatographic*). Square glass chromatography jar, 30 × 30 × 60 centimeters, designed for descending chromatography. The bottom of the tank is filled with 1.5 inches of a mixture of 70 percent *n*- propyl alcohol and 30 percent distilled water (v/v) and allowed to equilibrate for 2 days. The mobility of the capreomycin factors, capreomycin I and capreomycin II, depends to a large extent upon the amount of water vapor present in the chromatographic chamber. The mobility can be restricted by using more *n*-propyl alcohol and less water in the equilibrating solvent, or it can be increased by raising the water content. The R_f of value (the distance traveled by a particular antibiotic factor divided by the distance traveled by the solvent front) should be approximately 0.50 for capreomycin I and approximately 0.60 for capreomycin II.

(ii) *Preparation of solutions*—(*a*) *0.1N citrate buffer, pH 6.2.* Dissolve 21.0 grams of citric acid monohydrate in 1 liter of distilled water. Adjust the pH to 6.2 with 50 percent aqueous sodium hydroxide.

(*b*) *Developing solvent.* Mix *n*- propyl alcohol, distilled water, triethylamine, and glacial acetic acid in volumetric proportions of 75:33:8:8, respectively.

(iii) *Preparation of the capreomycin sample solution.* Dissolve approximately 200 milligrams of the sample, accurately weighed, with distilled water in a 10-milliliter volumetric flask. Dilute to volume with distilled water. This sample should be refrigerated when not in use.

(iv) *Preparation of the chromatogram.* Use separate sheets for each capreomycin sample solution and for blanks without sample application. Evenly apply a 100-microliter aliquot of the capreomycin sample solution to the origin line of a sheet. A U-shaped glass rod is placed under the chromatogram during spotting. Dry the streak thoroughly with warm air. Place the sample sheets and a blank sheet in the chamber and develop them in a descending manner for 16 hours. Remove the sheets from the chamber and air dry for about 1 hour.

(v) *Processing the chromatogram.* Examine each sheet under short-wavelength (254 nanometers) ultraviolet light and locate the main streak (R_f approximately 0.5) and the preceding streak (capreomycin II, R_f approximately 0.6). Outline the main zone lightly with a pencil. Outline an area on the blank sheet approximately equal in size and in the same location as those outlined on the sample sheets. Cut the marked areas from the sheets and then cut them into approximately 1.5-centimeter squares. For each sheet, place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask.

(vi) *Elution.* To each flask, add 10 milliliters of 0.1*N* citrate buffer, pH 6.2, and agitate on a reciprocating shaker for 1 hour. Filter each of the shaken solutions through Whatman No. 1 filter paper into separate 10-milliliter glass-stoppered Erlenmeyer flasks. Transfer 3 milliliters of each filtrate into separate 50-milliliter volumetric flasks and dilute to volume with distilled water.

(vii) *Capreomycin sample solution for direct measurement of absorbance.* Pipette 1.0 milliliter of the sample solution prepared as described in paragraph (b)(8)(iii) of this section into a 100-milliliter glass-stoppered volumetric flask. Dilute to volume with 0.1*N* citrate buffer, pH 6.2. Transfer 3.0 milliliters of this solution into a 50-milliliter volumetric flask and dilute to volume with distilled water.

(viii) *Absorbance measurement.* Using a suitable spectrophotometer, 1.0-centimeter quartz cells, and distilled water as the reference solvent, determine the absorbance of each eluate and of each sample solution at the absorption maximum at about 268 nanometers.

(ix) *Calculation of percent capreomycin I in samples.* Calculate as follows:

$$\text{Percent capreomycin I} = \frac{A_I - A_B}{A_s} \times 100$$

where:

A_I=Absorbance of the eluate from the main zone of the sample sheet;

A_B=Absorbance of the eluate from the area of the blank sheet corresponding to the area of the capreomycin I of the sample sheet;

A_s=Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

If the assay of capreomycin I from the chromatogram is less than 90 percent of total capreomycins, repeat the procedure described in paragraph (b)(8)(iv), (v), (vi), (vii), and (viii) of this section two more times and at the same time determine the recovery of total capreomycins from the unchromatographed sheet as described in paragraph (b)(8)(x) of this section. The average of three valid assays should then be reported.

(x) *Recovery of total capreomycins from the unchromatographed sheet*—(*a*) *Procedure.* Evenly apply a 100-microliter aliquot of the capreomycin sample solution (prepared as described in paragraph (b)(8)(iii) of this section) to the origin line of a sheet. Dry the streak thoroughly with warm air. The paper is not chromatographed before elution. Cut the area containing the streak from the sheet and then cut into approximately 1.5-centimeter squares. Place the squares into a glass-stoppered 50-milliliter Erlenmeyer flask and proceed as directed in paragraph (b)(8) (vi), (vii), and (viii) of this section. Likewise, cut an equal-sized area from an untreated part of the sheet and cut it into approximately 1.5-centimeter squares. Place the squares

in a glass-stoppered 50-milliliter Erlenmeyer flask and also proceed as directed in paragraph (b)(8) (vi), (vii), and (viii) of this section.

(*b*) *Calculation.* Calculate the recovery of total capreomycins as follows:

$$\text{Recovery of total capreomycins} = \frac{At - Ab}{A_s} \times 100$$

where:

At=Absorbance of the eluate from the unchromatographed sheet;

Ab=Absorbance of the eluate from the unchromatographed blank sheet;

As=Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

To be a valid assay, the recovery of total capreomycins from the unchromatographed sheet must be 100±2 percent.

(9) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except ignite at 700° C.

(10) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 448.20a Sterile colistimethate sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistimethate sodium is the sodium salt of a kind of colistin methane sulfonate or a mixture of two or more such salts. It is a white to slightly yellow, odorless, fine powder which is freely soluble in water. It is so purified and dried that:

(i) Its potency is not less than 390 micrograms of colistin base equivalent per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin base equivalent that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) It gives a positive identity test for colistimethate sodium.

(viii) It passes the test for free colistin.

(ix) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, identity, free colistin, and heavy metals.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 containers, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 vials or if each vial contains less than 150 milligrams of colistimethate, a minimum of 60 vials.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repacking or for use in manufacturing another drug, dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10-percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute the

stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin base equivalent per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 10 milligrams of colistin base equivalent per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using a 1-percent aqueous solution prepared in the following manner: Weigh accurately 0.5 gram of sample and transfer to a 125-milliliter Erlenmeyer flask. Add 50 milliliters of freshly boiled distilled water, stopper, and shake until the sample is in solution.

(7) *Identity*—(i) *Infrared.* Proceed as directed in §436.211 of this chapter, using a 1-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(ii) *Iodine reduction.* Dissolve 40 milligrams of sample in 1.0 milliliter of 1.0*N* hydrochloric acid and add 0.5 milliliter of 0.02*N* iodine. The color is rapidly discharged.

(8) *Free colistin.* Dissolve 80 milligrams of sample in 3.0 milliliters of distilled water and add 0.05 milliliter of 10 percent w/v solution of silicotungstic acid. It passes the test for free colistin if no immediate precipitate is produced.

(9) *Heavy metals.* Proceed as directed in §436.208 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 44 FR 10381, Feb. 20, 1979; 44 FR 22059, Apr. 13, 1979; 50 FR 19920, May 13, 1985]

§448.21 Colistin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate is the white to slightly yellow, odorless sulfate salt of a kind of colistin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 500 micrograms of colistin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 7.0.

(v) It gives a positive identity test for colistin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b).

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* To about 20 milligrams of sample, add 2.0 milliliters of pH 7.0 buffer (prepared by adding 29.63 milliliters of 1 N sodium hydroxide to 50 milliliters of 1 M potassium dihydrogen phosphate, adjusting to pH 7.0 if necessary, and diluting to 100 milliliters with distilled water) and 0.2 milliliter of a 0.5 percent aqueous triketohydrindene hydrate solution, and bring to boil. A purple color is produced.

[39 FR 19115, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 448.23 Cyclosporine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclosporine is a cyclic polypeptide consisting of 11 amino acids. It is a white or essentially white finely crystalline powder. It is so purified and dried that:

(i) Its cyclosporine content is not less than 975 micrograms per milligram and not more than 1,020 micrograms per milligram on the anhydrous basis.

(ii) Its loss on drying is not more than 3.0 percent.

(iii) Its heavy metals content is not more than 20 parts per million.

(iv) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cyclosporine content, loss on drying, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Cyclosporine content.* Proceed as directed in § 436.346 of this chapter, except prepare the working standard and sample solutions and calculate the cyclosporine content as described in paragraphs (b)(1) (i) and (ii) of this section. A typically suitable column for cyclosporine is a 250-millimeter column having an inside diameter of 4 millimeters packed with octyl silane chemically bonded to totally porous microsilica particles, 5 to 7 microns in diameter.

(i) *Preparation of working standard and sample solutions.*

NOTE: Dissolve working standards and samples immediately before analysis.

(*a*) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the working standard in ethanol by shaking for at least 15 minutes. If necessary, ultrasonicate until the solution becomes completely clear. Dilute with ethanol to obtain a solution containing 1,000 micrograms of cyclosporine activity per milliliter.

(*b*) *Preparation of sample solutions.* Prepare all sample solutions as directed for preparation of working standard solutions, except dilute with ethanol to obtain a solution containing 1,000 micrograms of cyclosporine per milliliter (estimated).

(ii) *Calculations.* Calculate the micrograms of cyclosporine per milligram of sample as follows:

$$\begin{array}{c}\text{Micrograms of}\\ \text{cyclosporine}\\ \text{per milligram}\end{array} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cyclosporine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cyclosporine peak in the chromatogram of the cyclosporine working standard;

P_s=Cyclosporine activity in the cyclosporine working standard solution in micrograms per milliliter;

C_u=Milligrams of cyclosporine per milliliter of sample solution; and

m=Percent loss on drying of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(4) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraphs (b)(1) of this section compares qualitatively to that of the cyclosporine working standard.

[49 FR 22632, May 31, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§ 448.25 Gramicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gramicidin is the white, or nearly white, odorless, crystalline compound of a kind of gramicidin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It has a potency of not less than 900 micrograms of gramicidin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 3 percent.

(iv) Its residue on ignition is not more than 1.0 percent.

(v) Its melting point is not below 229° C after drying in vacuum at 60° C for 3 hours.

(vi) When calculated on the anhydrous basis, the difference between the absorptivity value at the maximum occurring at 282 nanometers and the absorptivity value at the minimum occurring at 247 nanometers is 100±4 percent of the difference obtained with the gramicidin working standard.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, residue on ignition, melting point, identity, and crystallinity.

(ii) Samples required of the batch: Ten packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient alcohol U.S.P. XX to obtain a stock solution of convenient concentration. Further dilute the stock solution volumetrically with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(6) *Identity.* Accurately weigh about 20 milligrams of the sample and dilute in ethyl alcohol to give a concentration of 0.05 milligram (estimated) of gramicidin per milliliter. Prepare a solution of the gramicidin working standard to contain 0.05 milligram per milliliter in ethyl alcohol. Using a suitable recording spectrophotometer with 1-centimeter cells, record the ultraviolet absorbance spectrum of each solution from 220 nanometers to 320 nanometers. The ultraviolet absorbance spectrum of the sample solution should compare qualitatively to that of the working standard solution. Determine the absorptivities of each at the maximum occurring at 282 nanometers and at the minimum occurring at 247 nanometers (the exact position of the maximum and minimum of the gramicidin working standard should be determined for the particular instrument used).

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19115, May 30, 1974, as amended at 41 FR 24883, June 21, 1976; 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 448.30 Polymyxin B sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH is an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) It gives positive color identity tests for polymyxin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Add 2.0 milliliters of sterile distilled water to each 5 milligrams of an accurately weighed portion of the sample. Dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution containing 10,000 units of polymyxin B

per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Identity.* (i) To a solution of 2 milligrams of the sample in 5.0 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine. Boil for 1 minute and cool. A blue color is a positive test.

(ii) To a solution of 2 milligrams of the sample in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10) and mix well. Add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop. A reddish-violet color is a positive test.

[40 FR 22253, May 22, 1975, as amended at 50 FR 19920, May 13, 1985]

§ 448.30a Sterile polymyxin B sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its residue on ignition is not more than 5 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 100 parts per million.

(ix) It gives positive color identity tests for polymyxin.

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, if the drug is packaged for dispensing its labeling shall bear the statement, ''Caution: This drug should be given intramuscularly and/or intrathecally only to hospitalized patients so as to provide constant supervision by a physician''.

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, heavy metals, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the drug is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers plus one additional package containing 1 gram of the batch.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water for each 5 milligrams of weighed sample. Further dilute an aliquot of this solution with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion

from each container. Dilute with sufficient solution 6 to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20,000 units of polymyxin B per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Identity.* (i) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops; and

(ii) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of cupric sulfate solution (1:100), mixing after the addition of each drop; a reddish-violet color is produced.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16683, Mar. 13, 1981; 46 FR 22359, Apr. 17, 1981; 50 FR 19920, May 13, 1985]

§ 448.75 Tyrothricin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tyrothricin is a white to brownish-white compound of a kind of tyrothricin or a mixture of two or more such compounds. It consists principally of gramicidin and tyrocidine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms and not more than 1,400 micrograms of tyrothricin per milligram.

(ii) Its loss on drying is not more than 5 percent.

(iii) It gives a positive identity test for tyrothricin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, and identity.

(ii) Samples required: five packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to give a stock solution of convenient concentration. Further dilute the stock solution with 95 percent ethyl alcohol, U.S.P. XVIII or equivalent, to the reference concentration of 0.20 microgram of tyrothricin per milliliter (estimated). Average the absorbance values for the tyrothricin sample and read the gramicidin concentration from the gramicidin standard response line. Multiply by 5 to obtain the number of micrograms of tyrothricin in the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Identity.* To 5 milliliters of *p*-dimethylaminobenzaldehyde (T.S.) add about 5 milligrams of tyrothricin. Shake well for 2 minutes; then add 2 drops of 0.1*M* sodium nitrite and 5 milliliters of water. A blue color is produced.

Subpart B—Oral Dosage Forms

§ 448.121 Colistin sulfate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for oral suspension is a dry mixture of colistin sulfate, with or without one or more suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings. The colistin sulfate content is 5.0 milligrams of colistin per milliliter of the reconstituted suspension. Its potency

is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain. The loss on drying is not more than 3.0 percent. The pH of the reconstituted suspension is not less than 5.0 and not more than 6.0. The colistin sulfate used conforms to the standards prescribed by § 448.21(a)(1).

(2) *Labeling.* It shall be labeled as prescribed in § 432.5 of this chapter,

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the suspension as directed in the label. Remove an accurately measured representative portion of the reconstituted suspension with a hypodermic needle and syringe and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension when reconstituted as directed in the labeling.

[39 FR 19115, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 448.123 Cyclosporine oral dosage forms.

§ 448.123a Cyclosporine oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclosporine oral solution contains, in each milliliter, 100 milligrams of cyclosporine in a suitable and harmless alcohol-vegetable oil solution. Its cyclosporine content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cyclosporine that it is represented to contain. The cyclosporine used conforms to the standards prescribed by § 448.23, except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cyclosporine used in making the batch for cyclosporine content, loss on drying, and identity.

(*b*) The batch for cyclosporine content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The cyclosporine used in making the batch: Six packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; cyclosporine content.* Proceed as directed in § 436.346 of this chapter, except prepare the working standard and sample solutions and calculate the cyclosporine content as described in paragraphs (b) (1) and (2) of this section. A typically suitable column for cyclosporine dosage forms is 250 millimeters long having an inside diameter of 4 millimeters packed with dimethyl silane chemically bonded to porous silica particles 10 microns in diameter [RP–2 (E.M. Science, S. Plainfield, NJ)].

(1) *Preparation of working standard and sample solutions.*

NOTE: Prepare working standard and sample solutions immediately before analysis.

(i) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the working standard in ethanol by shaking for at least 15 minutes. If necessary, ultrasonicate until the solution becomes completely clear. Dilute with ethanol to obtain a solution containing 1 milligram of cyclosporine activity per milliliter.

(ii) *Preparation of sample solution.* Place an accurately measured representative volume of the cyclosporine oral solution into a volumetric flask. Add sufficient ethanol to obtain a concentration of 1 milligram of cyclosporine activity per milliliter (estimated).

(2) *Calculations.* Calculate the cyclosporine content as follows:

$$\text{Milligrams of cyclosporine per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cyclosporine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cyclosporine peak in the chromatogram of the cyclosporine working standard;

P_s=Cyclosporine activity of the cyclosporine working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

[49 FR 22633, May 31, 1984, as amended at 55 FR 11584, Mar. 29, 1990. Redesignated at 55 FR 19873, May 14, 1990]

§448.123b Cyclosporine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclosporine capsules are composed of cyclosporine in a suitable and harmless alcohol-vegetable oil solution enclosed by a soft gelatin capsule. Its cyclosporine content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cyclosporine that it is represented to contain. The capsules shall disintegrate within 30 minutes. The cyclosporine used conforms to the standards prescribed by §448.23, except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The cyclosporine used in making the batch for cyclosporine content, loss on drying, and identity.

(B) The batch for cyclosporine content and disintegration time.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The cyclosporine used in making the batch: Six packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 36 capsules.

(b) *Test and methods of assay*—(1) *Cyclosporine content.* Proceed as directed in §436.346 of this chapter, except prepare the working standard and sample solutions and calculate the cyclosporine content as described in paragraphs (b)(1)(i) (A) and (B) and (b)(1)(ii) of this section. A typically suitable column for cyclosporine dosage forms is 250 millimeters long having an inside diameter of 4 millimeters packed with dimethyl silane chemically bonded to porous silica particles 10 microns in diameter (RP–2 (E.M. Science, S. Plainfield, NJ)).

(i) *Preparation of working standard and sample solutions.*

NOTE: Prepare working standard and sample solutions immediately before analysis.

(A) *Working standard solution.* Dissolve an accurately weighed portion of the working standard in ethanol by shaking for at least 15 minutes. If necessary, ultrasonicate until the solution becomes completely clear. Dilute with ethanol to obtain a solution containing 1 milligram of cyclosporine activity per milliliter.

(B) *Sample solution.* Cut open a representative number of capsules with a sharp blade and quantitatively transfer the capsule contents to a volumetric flask. Add sufficient ethanol to obtain a concentration of 1 milligram of cyclosporine activity per milliliter (estimated).

(ii) *Calculations.* Calculate the cyclosporine content in milligrams per capsule as follows:

$$\text{Milligrams of cyclosporine per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the cyclosporine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cyclosporine peak in the chromatogram of the cyclosporine working standard;

P_s=Cyclosporine content in the cyclosporine working standard solution in micrograms per milliliter;
d=Dilution factor of the sample; and
n=Number of capsules in the sample assayed.

(2) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in § 436.212(e)(5).

[55 FR 19873, May 14, 1990; 55 FR 22014, May 30, 1990]

Subpart C—Injectable Dosage Forms

§ 448.210 Sterile bacitracin.

The requirements for certification and the tests and methods of assay for sterile bacitracin packaged for dispensing are described in § 448.10a.

§ 448.215 Sterile capreomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile capreomycin sulfate packaged for dispensing are described in § 448.15a.

§ 448.220 Colistimethate sodium injectable dosage forms.

§ 448.220a Sterile colistimethate sodium.

The requirements for certification and the tests and methods of assay for sterile colistimethate sodium packaged for dispensing are described in § 448.20a.

§ 448.223 Cyclosporine for infusion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclosporine for infusion is a solution of cyclosporine in a suitable and harmless alcohol derivatized vegetable oil vehicle. Its cyclosporine content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of cyclosporine that it is represented to contain. It is sterile. It contains not more than 42 endotoxin units per milliliter (United States Pharmacopeia endotoxin units). The cyclosporine used conforms to the standards prescribed by § 448.23.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The cyclosporine used in making the batch for cyclosporine content, loss on drying, heavy metals, and identity.

(*b*) The batch for cyclosporine content, sterility, and bacterial endotoxins.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The cyclosporine used in making the batch: Six packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Cyclosporine content.* Proceed as directed in § 436.346 of this chapter, except prepare the working standard and sample solutions and calculate the cyclosporine content as described in paragraphs (b)(1) (i) and (ii) of this section. A typically suitable column for cyclosporine dosage forms is 250 millimeters long having an inside diameter of 4 millimeters packed with dimethyl silane chemically bonded to porous silica particles 10 microns in diameter [RP–2 (E.M. Science, S. Plainfield, NJ)].

(i) *Preparation of working standard and sample solutions.*

NOTE: Prepare working standard and sample solutions immediately before analysis.

(*a*) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the working standard in ethanol by shaking for at least 15 minutes. If necessary, ultrasonicate until the solution becomes completely clear. Dilute with ethanol to obtain a solution containing 1 milligram of cyclosporine activity per milliliter.

(*b*) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the concentration of cyclosporine in a given volume of the resultant preparation, remove an

accurately measured portion from each container. Dilute with ethanol to obtain a stock solution of 1 milligram of cyclosporine activity per milliliter (estimated).

(ii) *Calculations.* Calculate the cyclosporine content of the vial as follows:

$$\text{Milligrams of cyclosporine per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the cyclosporine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cyclosporine peak in the chromatogram of the cyclosporine working standard;

P_s=Cyclosporine activity in the cyclosporine working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia XX bacterial endotoxins test.

[49 FR 22633, May 31, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§448.230 Sterile polymyxin B sulfate.

The requirements for certification and the tests and methods of assay for sterile polymyxin B sulfate packaged for dispensing are described in §448.30a.

[44 FR 10379, Feb. 20, 1979]

Subpart D—Ophthalmic Dosage Forms

§448.310 Bacitracin ophthalmic dosage forms.

§448.310a [Reserved]

§448.310b Bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment contains bacitracin, neomycin sulfate, and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains 500 units of bacitracin, 3.5 milligrams of neomycin, and 10,000 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin used conforms to the standards prescribed by §448.10a(a)(1), except pyrogens, residue on ignition, and heavy metals. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) of this chapter, except pyrogens. The polymixin B sulfate used conforms to the standards prescribed by §448.30a(a)(1), except pyrogens, residue on ignition, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*d*) The batch for bacitracin content, neomycin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The polymixin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*d*) The batch:

(*1*) For all tests except sterility: A minimum of 17 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assays*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed for bacitracin zinc in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and futher dilute with solution 6 to the reference concentration of 10 units of Polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[42 FR 27230, May 27, 1977, as amended at 47 FR 23442, May 28, 1982; 50 FR 19920, May 13, 1985]

§ 448.310c Bacitracin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strenght, quality, and purity.* Bacitracin ophthalmic ointment contains bacitracin in a suitable and harmless ointment base. Each gram contains 500 units of bacitracin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin used conforms to the standards prescribed by § 448.10a(a)(1), except pyrogens, residue on ignition, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin zinc in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in §436.206 of this chapter.

[42 FR 27230, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§448.313 Bacitracin zinc ophthalmic dosage forms.

§448.313a Bacitracin zinc-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate ophthalmic ointment contains in each gram 500 units of a bacitracin and 10,000 units of polymyxin B in a suitable and harmless ointment base. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin zinc used conforms to the standards prescribed by §448.13a(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 17 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochoric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the final sample solution.) Remove an aliquot and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6) and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[42 FR 27231, May 27, 1977, as amended at 49 FR 34351, Aug. 30, 1984; 50 FR 19920, May 13, 1985]

§ 448.313b Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ophthalmic ointment; bacitracin zinc-neomycin sulfate-polymyxin B sulfate-hydrocortisone ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ophthalmic ointment is bacitracin zinc, neomycin sulfate, and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains:

(i) 400 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B with or without 10 milligrams of hydrocortisone acetate;

(ii) 500 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B; or

(iii) 400 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 10 milligrams of hydrocortisone.

Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin zinc used conforms to the standards prescribed by § 448.13a(a)(1). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) of this chapter, except pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1), except

pyrogens, residue on ignition, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*d*) the batch for bacitracin content, neomycin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*d*) The batch:

(*1*) For all tests except sterility: A minimum of 17 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extration procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the 1.0 unit per milliliter sample solution.) Remove an aliquot and further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1) to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of 3 more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further

dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[42 FR 27231, May 27, 1977, as amended at 47 FR 23443, May 28, 1982; 48 FR 21564, May 13, 1983; 50 FR 19920, May 13, 1985]

§ 448.321 Colistin sulfate for ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for ophthalmic solution is a dry mixture of colistin sulfate and mannitol packaged in combination with a suitable and harmless diluting solution which contains buffers and a preservative. When reconstituted as directed in the labeling, each milliliter contains 1.2 milligrams of colistin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain. It is sterile. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 6.3. The colistin sulfate used conforms to the standards prescribed by § 448.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Remove an accurately measured representative portion of the reconstituted solution and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution reconstituted as directed in the labeling.

[39 FR 19115, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 448.330 Polymyxin B sulfate-trimethoprim hemisulfate ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate-trimethoprim hemisulfate ophthalmic solution contains, in each milliliter, 10,000 units of polymyxin B and 1.0 milligram of trimethoprim in a suitable and harmless isotonic aqueous vehicle. It contains suitable and harmless buffers and preservatives. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its trimethoprim content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of trimethoprim that it is represented to contain. It is sterile. Its pH is not less than 3.0 and not more than 5.5. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(B) The trimethoprim used in making the batch for all U.S.P. specifications.

(C) The batch for polymyxin B content, trimethoprim content, sterility, and pH.

(ii) Samples if required by the Director, Center for Drug Evaluation and Research:

(A) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The trimethoprim hemisulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(C) The batch:

(*1*) For all tests except sterility: A minimum of 7 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Polymyxin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 10 percent potassium phosphate buffer, pH 6.0 (solution 10), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Trimethoprim content.* Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, and a column packed with octyl, octadecyl, or phenyl groups chemically bonded to porous silica ranging from 3 to 10 micrometers in particle size. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements and calculations are as follows:

(i) *Reagents*—(A) *Diluting fluid.* 13 percent acetonitrile in 0.01*M* hydrochloric acid.

(B) *Mobile phase.* Mix 0.015*M* ethanesulfonic acid in acetonitrile: water (130:870) and adjust to pH 3.5 with 50 percent w/w sodium hydroxide and hydrochloric acid solution. Filter the mobile phase through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Place approximately 40 milligrams of trimethoprim working standard, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with 13 percent acetonitrile in 0.01*M* hydrochloric acid, and mix. Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, and dilute to volume with 13 percent acetonitrile in 0.01*M* hydrochloric acid, and mix.

(B) *Sample solution.* Transfer 4.0 milliliters of the sample into a 50-milliliter volumetric flask and dilute to volume with 13 percent acetonitrile in 0.01*M* hydrochloric acid.

(C) *Resolution test solution.* Place approximately 40 milligrams of trimethoprim working standard and 15 milligrams of 2-amino-4-hydroxy-5-(3′,4′5′)-trimethoxybenzyl pyrimidine, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with 13 percent acetonitrile in 0.01*M* hydrochloric acid, and mix. Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, and dilute to volume with 13 percent acetonitrile in 0.01*M* hydrochloric acid, and mix. Prepare the resolution test solution just prior to its introduction into the chromatograph pumping system.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_s is satisfactory if it is not more than 1.4 at 10 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 1,500 theoretical plates.

(C) *Resolution.* The resolution (R) between 2-amino-4-hydroxy-5-(3′,4′,5′)-trimethoxybenzyl pyrimidine (AHTP) and trimethoprim is satisfactory if it is not more than 1.5.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of five replicate injections is satisfactory if it

is not more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the milligrams of trimethoprim per milliliter of sample as follows:

$$\text{Milligrams of trimethoprim per milliliter} = \frac{A_u \times W_s \times 0.8555}{A_s \times 40}$$

where:

A_u=Area of the trimethoprim peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the trimethoprim peak in the chromatogram of the trimethroprim working standard;

W_s=Weight of the trimethoprim working standard in milligrams; and

0.855=Gravimetric conversion factor trimethoprim hemisulfate to trimethoprim.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[54 FR 38376, Sept. 18, 1989, as amended at 59 FR 8399, Feb. 22, 1994]

Subpart E—Otic Dosage Forms

§ 448.421 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension is a suspension containing colistin sulfate, neomycin sulfate, thonzonium bromide, and hydrocortisone acetate, and one or more preservatives, dispersing agents, and buffer substances. Each milliliter contains 3.0 milligrams of colistin, 3.3 milligrams of neomycin, 0.5 milligram of thonzonium bromide, and 10 milligrams of hydrocortisone acetate. Its content of colistin is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of colistin per milliliter that it is represented to contain. Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin per milliliter that it is represented to contain. It is sterile. Its pH is not less than 4.8 and not more than 5.2. The colistin sulfate used conforms to the standards prescribed therefor by § 448.21(a)(1). The neomycin sulfate used conforms to the standards prescribed in § 444.42a(a)(1)(i), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for colistin content, neomycin content, sterility, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of six immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Colistin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Thoroughly mix the sample and transfer an accurately measured representative portion of the sample into a 100-milliliter volumetric flask. Fill the flask to mark with 10 percent potassium phosphate buffer, pH 6.0 (solution 6). Further dilute with solution 6 to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

Thoroughly mix the sample and transfer an accurately measured representative portion into a 100-milliliter volumetric flask. Fill the flask to mark with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3). Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except transfer 0.25 milliliter of sample in lieu of 1 milliliter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[39 FR 19115, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§448.430 Polymyxin B sulfate-hydrocortisone otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate-hydrocortisone otic solution contains in each milliliter 10,000 units of polymyxin B and 5 milligrams of hydrocortisone in a suitable and harmless vehicle. Its polymyxin B sulfate content is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 3.0 and not more than 5.0. The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain the following:

(i) Results of tests and assays on—

(*a*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity; and

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample (usually 1.0 milliliter) with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except if the steroid prevents solubilization, use 0.25 milliliter of the sample in lieu of 1 milliliter and proceed as directed in paragraph (e)(2) of that section.

(3) *pH.* Proceed as directed in §436.202 of this chapter using the undiluted solution.

[44 FR 5880, Jan. 30, 1979, as amended at 50 FR 1504, Jan. 11, 1985; 50 FR 19920, May 13, 1985]

Subpart F—Dermatologic Dosage Forms

§448.510 Bacitracin dermatologic dosage forms.

§448.510a Bacitracin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin ointment is composed of 500 units of bacitracin per gram in a suitable ointment base. It may contain a suitable local anesthetic. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin used conforms to the standards described by §448.10(a)(1).

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by §432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin zinc in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1) and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[42 FR 27232, Nov. 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§§ 448.510b—448.510c [Reserved]

§ 448.510d Bacitracin-neomycin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-neomycin sulfate ointment contains bacitracin and neomycin sulfate in a suitable ointment base. Each gram contains 500 units of bacitracin and 3.5 milligrams of neomycin. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. The moisture content is not more than 0.5 percent. The bacitracin used conforms to the standards prescribed by §448.10(a)(1). The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1) of this chapter.

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by §432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safety and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, neomycin content, and moisture.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed for bacitracin zinc in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25 milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution. 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 27233, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.510e Bacitracin-neomycin sulfate-polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-neomycin sulfate-polymyxin B sulfate ointment, in a suitable and harmless ointment base, contains in each gram the following:

(i) 500 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B.

It may contain a suitable local anesthetic. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin used conforms to the standards prescribed by § 448.10(a)(1). The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1) of this chapter. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*d*) The batch for bacitracin content, neomycin content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The polymxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*d*) The batch: A minimum of 7 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed for bacitracin zinc in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 27233, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.510f Bacitracin-polymyxin B sulfate topical aerosol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin-polymyxin B sulfate topical aerosol is bacitracin and polymyxin B sulfate in a suitable and harmless vehicle, packaged in a pressurized container with a suitable and harmless inert gas. Each gram contains 500 units of bacitracin and 5,000 units of polymyxin B. It may contain a suitable local anesthetic. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent

of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin used conforms to the standards prescribed by § 448.10(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark;

(*b*) The name and quantity of each active ingredient contained in the drug; and

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potentcy, loss on drying, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The bacitracin used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing 1.0 gram.

(*c*) The batch: A minimum of 12 immediate containers.

(b) *Tests and methods of assay.* The container must remain inverted throughout the sampling procedure. Freeze the container overnight at −70 °C. Remove from the freezer and puncture the container to allow the propellant to dissipate. Open the container, mix well, and proceed as described in paragraphs (b) (1) and (2) of this section.

(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample from the container into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard, and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed portion of the sample from the container into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed § 436.201 of this chapter, using the titration procedure and calculation in paragraph (e)(3) of that section and 1- to 2-milliliter portions of the sample from the container.

[51 FR 35212, Oct. 2, 1986, as amended at 55 FR 9722, Mar. 15, 1990; 55 FR 11584, Mar. 29, 1990]

§ 448.513 Bacitracin zinc dermatologic dosage forms.

§ 448.513a Bacitracin zinc-polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate ointment contains bacitracin zinc and polymyxin B sulfate in a suitable and harmless ointment base. It may contain a suitable local anesthetic. Each gram contains 500 units of bacitracin and 10,000 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin zinc used conforms to the standards prescribed by § 448.13(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing equal portions of approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(*a*) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient addition hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the 1.0 unit per milliliter sample solution.) Remove an aliquot and further dilute with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(*b*) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient solution 1 to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows:

(a) If the ointment is not water miscible. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[42 FR 27234, May 27, 1977, as amended at 55 FR 50173, Dec. 5, 1990]

§448.513b Bacitracin zinc-neomycin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-neomycin sulfate ointment contains bacitracin zinc and neomycin sulfate in a suitable and harmless ointment base. Each gram contains 500 units of bacitracin and 3.5 miligrams of neomycin. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin zinc used conforms to the standards prescribed by §448.13(a)(1). The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1) of this chapter.

(2) *Labeling*—(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by §432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, neomycin content, and moisture.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows:

(*a*) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of

three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution will have the same amount of acid as the final sample solution.) Remove an aliquot and further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(*b*) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(a) If the ointment is not water miscible. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 27234, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.513c Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ointment; bacitracin zinc-neomycin sulfate-polymyxin B sulfate hydrocortisone ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* This drug, in a suitable and harmless ointment base, contains in each gram the following:

(i) 400 units of bacitracin, 3 milligrams of neomycin, and 8,000 units of polymyxin B; or

(ii) 400 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B, with or without 10 milligrams of hydrocortisone; or

(iii) 500 units of bacitracin, 3.5 milligrams of neomycin, and 5,000 units of polymyxin B; or

(iv) 500 units of bacitracin, 3.5 milligrams of neomycin, and 10,000 units of polymyxin B.

It may contain a suitable local anesthetic except for combinations in paragraph (a)(1)(ii) of this section that contain hydrocortisone. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more

than 0.5 percent. The bacitracin zinc used conforms to the standards prescribed by § 448.13(a)(1). The neomycin sulfate used conforms to the standards prescribed by § 444.42(a)(1) of this chapter. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* If it contains a steroid, it shall be labeled in accordance with the requirements of § 432.5 of this chapter. If it does not contain a steroid, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) The batch mark.

(ii) The name and quantity of each active ingredient contained in the drug.

(iii) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(iv) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*d*) The batch for bacitracin content, neomycin content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing 1.0 gram.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(d) The batch: A minimum of 7 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(*a*) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the 1.0 unit per milliliter sample solution.) Remove an aliquot and further dilute with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(*b*) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1) to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(*a*) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate

buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25- milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows:

(a) If the ointment is not water miscible. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 27235, May 27, 1977, as amended at 55 FR 50173, Dec. 5, 1990]

§ 448.513d Bacitracin zinc-polymyxin B sulfate topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate topical powder contains bacitracin zinc and polymyxin B sulfate in a suitable and harmless base. Each gram contains 500 units of bacitracin and 10,000 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 7.0 percent. It contains not more than an average of 10 microoganisms per gram. The bacitracin zinc used conforms to the standards prescribed by § 448.13(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling*—(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, polymyxin B content, moisture, and a microorganism count.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of 12 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Wash an accurately weighed sample (usually 2 grams) into a 100-milliliter volumetric flask with 0.01N hydrochloric acid. Dilute to volume with 0.01N hydrochloric acid. Further dilute an aliquot with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

NOTE: The final sample solution must contain the same amount of hydrochloric acid as the reference concentration of the working standard.

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed representative portion of the sample (usually 1 gram) in 20 milliliters of sterile distilled water. Wash into an appropriate-sized volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6). Further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Microorganism count*—(i) *Conduct of test for bacteria.* Using approximately 200 milligrams of powder from each of five separate immediate containers, proceed as directed in §436.20(e)(1) of this chapter, except after the three washings transfer the entire filter membrane to the surface of medium N as described in §436.20(c)(14) of this chapter. Incubate the plate for 7 days at 30° C. to 32° C. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

(ii) *Conduct of test for molds and yeasts.* Proceed as directed in §436.20(e)(1) of this chapter, using approximately 200 milligrams from each of the five containers tested, except transfer the entire filter membrane to the surface of medium N as described in §436.20(c)(14) of this chapter, and incubate at 22° C. to 25° C. for 7 days. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

[42 FR 27236, May 27, 1977, as amended at 50 FR 15110, Apr. 17, 1985; 55 FR 11584, Mar. 29, 1990]

§448.513e Bacitracin zinc-polymyxin B sulfate topical aerosol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate topical aerosol is bacitarcin zinc, polymyxin B sulfate in a suitable and harmless vehicle, packaged in a pressurized container with suitable and harmless inert gases. Each gram contains 120 units of bacitracin and 2,350 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 0.5 percent. It contains not more than an average of 10 microorganisms per container. The bacitracin zinc used conforms to the standards prescribed by §448.13(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1).

(2) *Labeling.* (i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, polymyxin B content, moisture, and a microorganism count.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of 12 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Sample preparation.* Spray, as directed in the labeling, the entire contents of each container to be tested into a separate 2-liter Erlenmeyer flask, held in a horizontal position. Add 500 milliliters of 0.01N hydrochloric acid and shake to dissolve the contents. Immediately remove aliquots of this sample solution and proceed as directed paragraph (b)(1)(i)(*a*) and (*b*) of this section for each antibiotic to be tested.

(*a*) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the sample solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

NOTE: The final sample solution must contain the same amount of hydrochloric acid as the reference concentration of the working standard.

(*b*) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the sample solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 10.0 units of polymyxin B per milliliter (estimated).

(ii) [Reserved]

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Microorganism count*—(i) *Conduct of test for bacteria.* Thoroughly cleanse the valve of each container to be tested with a suitable disinfectant. Into an empty, sterile Erlenmeyer flask, stoppered with a cotton plug, spray about one-half of the contents of each of five separate immediate containers by removing the cotton plug temporarily and using aseptic technique. Allow the propellant to evaporate. To the dry residue, which should not exceed 1 gram, add 500 milliliters of diluting fluid C as described in § 436.20(d)(3) of this chapter. Stopper the flask and swirl to dissolve the drug. As soon as the sample has completely dissolved, proceed as directed in § 436.20(e)(1)(ii) of this chapter, except after the three washings transfer the entire filter membrane to the surface of medium N as described in § 436.20(c)(14) of this chapter. Incubate the plate for 7 days at 30° C. to 32° C. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

(ii) *Conduct of test for molds and yeasts.* Proceed as directed in paragraph (b)(3)(i) of this section, except transfer the entire filter membrane to the surface of medium N as described in § 436.20(c)(14) of this chapter, and incubate at 25° C. for 7 days.

[42 FR 27237, May 27, 1977, as amended at 50 FR 15110, Apr. 17, 1985; 55 FR 11584, Mar. 29, 1990; 55 FR 40381, Oct. 3, 1990]

§ 448.513f Bacitracin zinc ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Bacitracin zinc ointment is composed of 500 units of bacitracin zinc per gram in a suitable ointment base. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its moisture content is not more than 0.5 percent. The bacitracin zinc used conforms to the standards prescribed by § 448.13(a)(1).

(2) *Labeling*—(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing 1.0 gram.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separtory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the aid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloic acid to each concentration of the standard response line so that each standard solution contains the same amout of acid as the 1.0 unit per milliliter sample solution.) Remove an aliquot and further dilute with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[42 FR 27237, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

Subpart G—Vaginal Dosage Forms [Reserved]

Subparts H–I [Reserved]

Subpart J—Certain Other Dosage Forms

§ 448.910 Bacitracin for prescription compounding.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin for prescription compounding is a white to brown, neutral, water-soluble polypeptide intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is so purified and dried that:

(i) Its potency is not less than 40 units of bacitracin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) Its pH in an aqueous solution containing 10,000 units per millilter is not less than 5.5 and not more than 7.5.

(v) It passes the identity test.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the United States Pharmacopeia (U.S.P.). It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 500,000 or 5 million units of bacitracin.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and

on the immediate container the following:

(i) The statement ''Caution: Federal law prohibits dispensing without prescription''.

(ii) The statement ''Not sterile''.

(iii) The batch mark.

(iv) The number of units of bacitracin activity in each milligram and the number of grams of bacitracin in the immediate container.

(v) The statement ''Expiration date _____'', the blank being filled in with the date that is 12 months after the month during which the batch was certified, unless the use of a longer dating period has been approved in accordance with § 432.5(a)(3) of this chapter.

(vi) The statement ''The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription''.

(vii) The statements, ''For use only in extemporaneous prescription compounding. Not for manufacturing use''.

(4) *Requests for certification; samples.* In addition to the requirements of § 432.1 of this chapter, each request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for bacitracin zinc in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Remove an aliquot of the stock solution, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10,000 units per milliliter.

(5) *Identity.* Proceed as directed in § 436.319 of this chapter.

[42 FR 27238, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§ 448.913 Bacitracin zinc for prescription compounding.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc for prescription compounding is the zinc salt of a kind of bacitracin or a mixture of two or more such salts intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is so purified and dried that:

(i) Its potency is not less than 40 units of bacitracin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) Its pH in a saturated aqueous solution is not less than 6.0 and not more than 7.5.

(v) Its zinc content is not more than 10 percent by weight on a moisture-free basis.

(vi) It passes the identity test.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the United States Pharmacopeia (U.S.P.). It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 500,000 or 5 million units of bacitracin.

(3) *Labeling.* Each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement ''Caution: Federal law prohibits dispensing without prescription''.

(ii) The statement ''Not sterile''.

(iii) The batch mark.

(iv) The number of units of bacitracin activity in each milligram of the bacitracin zinc, and the number of grams of bacitracin zinc in the immediate container.

(v) The statement ''Expiration date _____'', the blank being filled in with the date that is 12 months after the month during which the batch was certified, unless use of a longer dating period has been approved in accordance with the provisions of §432.5(a)(3) of this chapter.

(vi) The statement, ''The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription''.

(vii) The statements, ''For use only in extemporaneous prescription compounding. Not for manufacturing use''.

(4) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, zinc content, and identity.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample (usually 25 to 35 milligrams) in sufficient 0.01*N* hydrochloric acid to give a bacitracin concentration of 100 units per milliliter (estimated). Further dilute an aliquot with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

NOTE: The final sample solution must contain the same amount of hydrochloric acid as the reference concentration of the working standard.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a saturated solution (approximately 100 milligrams of the sample per milliliter).

(5) *Zinc content.* Proceed as directed in §436.312 of this chapter.

(6) *Identity.* Proceed as directed in §436.319 of this chapter.

[42 FR 27238, May 27, 1977, as amended at 50 FR 19920, May 13, 1985]

§448.930 Polymyxin B sulfate in certain other dosage forms.

§448.930a Polymyxin B sulfate for prescription compounding.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate for prescription compounding is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts intended for use in the extemporaneous compounding of prescriptions by practicing pharmacists. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) Its residue on ignition is not more than 5 percent.

(vi) It gives positive color identity tests for polymyxin.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 100 million units of polymyxin B.

(3) *Labeling.* In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement ''Caution: Federal law prohibits dispensing without prescription''.

(ii) The statement ''Not sterile''.

(iii) The batch mark.

(iv) The number of units of polymyxin B activity in each milligram of the polymyxin B sulfate and the number of grams of polymyxin B sulfate in the immediate container.

(v) The statement, ''The potency of this drug cannot be assured for longer than 60 days after the container is first opened for compounding a prescription''.

(vi) The statements, ''For use only in extemporaneous prescription compounding. Not for manufacturing use''.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, and identity.

(ii) Samples required: A 0.5-gram portion for each 5,000 packages in the batch, but in no case less than 10 such portions. Each such portion shall be collected at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water for each 5 milligrams of weighed sample. Further dilute an aliquot with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* (i) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops; and

(ii) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of a cupric sulfate solution (1:100) mixing after the addition of each drop; a reddish-violet color is produced.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

§ 448.930b Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile polymyxin B sulfate-benzalkonium chloride urethral lubricant is polymyxin B sulfate and benzalkonium chloride, with one or more suitable and harmless suspending agents, in a suitable and harmless base. It contains, in each gram, 5,000 units of polymyxin B and 330 micrograms of benzalkonium chloride. Its content of polymyxin B is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its pH is not less than 4.0 and not more than 5.5. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1), except sterility, pyrogens, and heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch.

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of

this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20(e)(1) of this chapter, except dissolve the ointment as follows: Aseptically transfer a portion of 0.25 gram from each of 10 immediate containers of the drug to 400 milliliters of diluting fluid D in an Erlenmeyer flask. Repeat the procedure on another 10 immediate containers. Swirl the flasks to dissolve the ointment.

(3) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

PART 449—ANTIFUNGAL ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
449.1—449.3 [Reserved]
449.4 Amphotericin B.
449.4a Amphotericin B for use in parenteral products.
449.10 Candicidin.
449.20 Griseofulvin.
449.40 Natamycin.
449.50 Nystatin.

Subpart B—Oral Dosage Forms

449.104 Amphotericin B oral suspension.
449.120 Griseofulvin oral dosage forms.
449.120a Griseofulvin tablets.
449.120b Griseofulvin capsules.
449.120c Griseofulvin oral suspension.
449.120d Griseofulvin (ultramicrosize) tablets.
449.150 Nystatin oral dosage forms.
449.150a Nystatin tablets.
449.150b Nystatin oral suspension.
449.150c Nystatin for oral suspension.
449.150d Nystatin pastilles.

Subpart C—Injectable Dosage Forms

449.204 Amphotericin B for injection.

Subpart D—Ophthalmic Dosage Forms

449.340 Natamycin ophthalmic suspension.

Subpart E [Reserved]

Subpart F—Dermatologic Dosage Forms

449.504 Amphotericin B dermatologic dosage forms.
449.504a Amphotericin B ointment.
449.504b Amphotericin B cream.
449.504c Amphotericin B lotion.
449.550 Nystatin dermatologic dosage forms.
449.550a Nystatin ointment.
449.550b Nystatin-iodochlorhydroxyquin ointment.
449.550c Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment; nystatin-neomycin sulfate-gramicidin-fludrocortisone acetate ointment.
449.550d Nystatin cream.
449.550e Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.
449.550f Nystatin topical powder.
449.550g Nystatin-neomycin sulfate-gramicidin topical powder.
449.550h Nystatin lotion.

Subpart G—Vaginal Dosage Forms

449.610 Candicidin vaginal dosage forms.
449.610a Candicidin vaginal ointment.
449.610b Candicidin vaginal tablets.
449.610c Candicidin vaginal capsules.
449.650 Nystatin vaginal dosage forms.
449.650a Nystatin vaginal tablets.
449.650b Nystatin vaginal suppositories.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19134, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§§449.1—449.3 [Reserved]

§449.4 Amphotericin B.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 15 percent of amphotericin A.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) It contains not more than 3.0 percent residue on ignition.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chapter, each package shall bear on its label the statements ''Store below 10° C.'' and ''Protect from light and moisture''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on the batch for potency, amphotericin A content, loss on drying, residue on iginition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot; dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content*—(i) *Amphotericin A.* Dry approximately 20 milligrams of the nystatin working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 200-milliliter volumetric flask. Add exactly 40.0 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(ii) *Amphotericin B.* Dry approximately 50 milligrams of the amphotericin B working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

The standard solution should be used for 1 day only.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(iv) *Blank.* Pipette 10 milliliters of dimethylsulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of nystatin standard, amphotericin B standard, and the sample at 304 nanometers and at 282 nanometers. Calculate the absorptivity of each standard at both wavelengths:

$$\text{Percent amphotericin A} = \frac{[(B \times S_2) - (b \times S_1)] \times 625}{W_s \times [(B \times a) - (b \times A)]}$$

where:

A=Absorptivity of nystatin standard at 282 nanometers;

B=Absorptivity of amphotericin B standard at 282 nanometers;
a=Absorptivity of nystatin standard at 304 nanometers;
b=Absorptivity of amphotericin B standard at 304 nanometers;
S_1=Absorbance of sample at 282 nanometers;
S_2=Absorbance of sample at 304 nanometers;
W_s=Weight of sample in grams (on an anhydrous basis).

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *Residue on ignition.* Proceed as directed in §436.207(a) of this chapter.

(6) *Identity.* Using the solutions prepared as described in paragraphs (b)(2)(ii), (iii), and (iv) of this section, record the absorption spectrum from 320 to 240 nanometers. Then dilute these solutions (1+9) with methyl alcohol and record the absorption spectrum from 400 to 320 nanometers. The sample exhibits absorption peaks at identical wavelengths with that of the amphotericin B standard. Depending on the amphotericin A content of the sample, a peak may occur at 304 nanometers.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 49 FR 2242, Jan. 19, 1984; 50 FR 19920, May 13, 1985]

§449.4a Amphotericin B for use in parenteral products.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 5 percent of amphotericin A.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) It contains not more than 0.5 percent residue on ignition.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by §432.5(b) of this chapter, each package shall bear on its label the statements ''Store below 10° C.'' and ''Protect from light and moisture''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, loss on drying, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content.* Proceed as directed in §449.4(b)(2).

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(5) *Residue of ignition.* Proceed as directed in §436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in §449.4(b)(7).

[39 FR 19134, May 30, 1974, as amended at 49 FR 2243, Jan. 19, 1984; 50 FR 19920, May 13, 1985]

§449.10 Candicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin is a brown to yellow powder. It is sparingly soluble in water; very slightly soluble in ethyl alcohol, butyl alcohol, and acetone. It is so purified and dried that:

(i) Its potency is not less than 1,000 micrograms of candicidin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 4 percent.

(iii) Its pH is not less than 8.0 nor more than 10.0 in a 1 percent aqueous suspension.

(iv) Its ultraviolet absorption spectrum is characteristic of a conjugated heptaene and is qualitatively the same as that of the candicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve a portion of the sample in sufficient dimethylsulfoxide to yield an estimated concentration of 1,000 micrograms of candicidin activity per milliliter. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin activity per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(4) *Identity*—(i) *Preparation of aqueous alcohol solution.* Prepare an aqueous alcohol solution by mixing 53 volumes of ethyl alcohol and 47 volumes of water.

(ii) *Preparation of standard solution.* Grind a small portion of the candicidin working standard to a fine powder with a mortar and pestle. Accurately weigh an amount equivalent to 20,000 micrograms of candicidin activity and transfer it to a 100-milliliter volumetric flask. Add about 50 milliliters of the aqueous alcohol solution and shake to effect complete dissolution. Bring to volume with the aqueous alcohol solution and mix well. Transfer a 25-milliliter aliquot to a 100-milliliter volumetric flask and bring to volume with the aqueous alcohol solution. This solution contains 50 micrograms of candicidin activity per milliliter.

(iii) *Preparation of sample solution.* Proceed as directed in paragraph (b)(4)(ii) of this section.

(iv) *Procedure.* Using a suitable recording spectrophotometer, record the absorption spectra of the standard solution and the sample solution between the wavelengths of 330 and 410 nanometers with the aqueous alcohol solution as the reference solution. Compare the absorption spectra of the standard solution and the sample solution. They should exhibit absorption maxima and minima at the same wavelengths, which are approximately 342, 359, 378, and 397 nanometers for the maxima and 348, 366, and 390 nanometers for the minima.

[39 FR 19134, May 30, 1974, as amended at 44 FR 30333, May 25, 1979; 49 FR 2243, Jan. 19, 1984]

§ 449.20 Griseofulvin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin is a microsize, white to pale-cream compound with the following chemical name: 7-chloro-2′,4,6-trimethoxy-6′β-methylspiro[benzofuran-2(3*H*),1′-[2]cyclohexene]-3,4′-dione. It is so purified and dried that:

(i) Its griseofulvin content is not less than 900 micrograms and not more than 1,050 micrograms of griseofulvin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its melting point, after drying, is not less than 217° C. and not more than 224° C.

(v) Its specific rotation in dimethylformamide at 25° C. is not less than +348° and not more than +364°.

(vi) Its ultraviolet absorption spectrum in methyl alcohol compares qualitatively with that of the griseofulvin reference standard.

(vii) Its residue on ignition is not more than 0.2 percent.

(viii) Its heavy metals content is not more than 25 parts per million.

(ix) Its specific surface area is not less than 1.3 and not more than 1.7 square meters per gram.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 1 gram.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(5) *Specific rotation.* Accurately weigh approximately 250 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of dimethylformamide. Bring to volume with dimethylformamide, stopper, and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(6) *Identity.* Dissolve an accurately weighed portion of the sample and of the griseofulvin working standard and dissolve each in sufficient methyl alcohol to obtain a concentration of 10 micrograms of griseofulvin per milliliter and mix well. (The standard solution can be kept under refrigeration and used for up to 1 month.) Record the ultraviolet absorption spectrum of solutions of the sample and standard from 240 to 320 nanometers. The spectral curves shall be similar, and each shall have a maximum at 292± 2 nanometers and a minimum at 269± 2 nanometers.

(7) *Residue on ignition.* Proceed as directed in § 436.207 of this chapter.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(9) *Specific surface area*—(i) *Procedure.* Determine the apparent particle size in microns by the air-permeation method, using a suitable subsieve sizer. Weigh 1.819 grams ±0.001 gram of the sample and transfer to the compression tube of the apparatus. Compact the sample with moderate pressure so that it has a uniform porosity. Pass compressed dry air through the sample and measure the air pressure with a water manometer. Observe the porosity and calculate the apparent particle size from the instrument equation or read it from a chart that has been calculated in accordance with the equation. Repeat the readings at successively higher degrees of compaction until the apparent particle size reaches a minimum. Calculate the observed specific surface area (SSA) in square meters per gram of sample, as follows:

$$\text{Observed SSA} = \frac{6}{\text{Minimum apparent particle size in microns} \times 1.455 \times F}$$

where F is a factor used to correct the apparent particle size to the true particle size:

Porosity reading	F
0.80	1.3771
0.76	1.4142
0.72	1.4573
0.68	1.5082
0.64	1.5690
0.60	1.6432
0.56	1.7353
0.52	1.8528
0.48	2.0076
0.44	2.2203
0.40	2.5298

(ii) *Standard.* Determine the observed specific surface area of the griseofulvin specific surface area standard by the method prescribed in paragraph (b)(9)(i) of this section, using the same instrument and the same air pressure setting.

(iii) *Calculations.* Calculate the corrected specific surface area of the sample as follows:

$$\text{SSA of sample} = \frac{\text{Observed SSA of sample} \times \text{assigned SSA of standard}}{\text{Observed SSA of standard}}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20660, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.40 Natamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Natamycin is 22-[(3 - amino - 3,6 - dideoxy -β-D-mannopyranosyl) - oxy] - 1,3,26-trihydroxy - 12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.0^{5,7}] octacosa-8,14,16,18,20 - pentaene-25-carboxylic acid. It is an off-white to cream colored powder which may contain up to 3 moles of water. It is practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of natamycin per milligram on an anhydrous basis.

(ii) Its moisture content is not less than 6.0 percent and not more than 9.0 percent.

(iii) Its pH in a 1 percent aqueous suspension is not less than 5.0 and not more than 7.5.

(iv) It passes the identity test.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay.* Dilute solutions of natamycin are very sensitive to light and should be kept in the dark as much as possible or substantial decomposition will take place.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in dimethylsulfoxide and further dilute with sufficient dimethylsulfoxide to give a concentration of 100 micrograms of natamycin per milliliter (estimated). Further dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 5.0 micrograms of natamycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1.0 percent aqueous suspension.

(4) *Identity.* Accurately weigh approximately 50 milligrams of the sample into a 200-milliliter volumetric flask. Add approximately 5.0 milliliters of distilled water, and completely moisten the sample. Then add approximately 100 milliliters of an acid-alcohol solvent (0.1 percent glacial acetic acid in methyl alcohol) and stir or shake mechanically in the dark until solution is complete. Dilute to volume with the acid-alcohol solvent. Transfer 2.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with the acid-alcohol solvent. Using a suitable spectrophotometer with 1-centimeter cells and the acid-alcohol solvent as a blank, record the ultraviolet absorption spectrum from 215 to 330 nanometers. The spectrum compares qualitatively to that of the natamycin working standard similarly treated.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 55384, Nov. 28, 1978, as amended at 46 FR 16684, Mar. 13, 1981]

§ 449.50 Nystatin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin is the yellow to light-tan compound of a kind of nystatin or a mixture of two or more such compounds. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. It is so purified and dried that:

(i) Its potency is not less than 4,400 units of nystatin per milligram; except, if it is packaged for extemporaneous preparation of oral suspensions, its potency is not less than 5,000 units of nystatin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) Its pH in a 3 percent aqueous suspension is not less than 6.5 and not more than 8.0.

(v) It passes the identity test.

(vi) If it is packaged for extemporaneous preparation of oral suspensions, it passes the suspendibility test.

(vii) If it is packaged for extemporaneous preparation of oral suspensions, it is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity. In addition, if it is packaged for extemporaneous preparation of oral suspensions, results of tests and assays on the batch for suspendibility and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylformamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 3 percent aqueous suspension of the drug.

(5) *Identity.* Weigh approximately 100 milligrams of the sample into a 200-milliliter, glass-stoppered, volumetric flask. Add 50 milliliters of absolute methyl alcohol and 10 milliliters of glacial acetic acid. When the sample has dissolved, dilute to volume with methyl alcohol. Transfer 2 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Use the same dilution of acetic acid in methyl alcohol as the blank. Immediately determine the absorption peaks at 230, 291, 305, and 319 nanometers, and the shoulders at 279±2 nanometers, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with the blank. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 nanometers to 350 nanometers. If a nonrecording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two absorbances

$$(A_{230}/A_{279})$$

should be not less than 0.90 and not more than 1.25.

(6) *Suspendibility test.* Transfer 200 milligrams of the sample into a 250-milliliter beaker containing 200 milliliters of water. Swirl the suspension gently with a stirring rod. Allow the beaker to remain still for 2 minutes and observe the bottom. It passes the test if the powder remains in suspension. If a significant amount of sediment is observed, withdraw an accurately measured aliquot of the undisturbed suspension and assay as directed in § 449.150c(b)(1) of this chapter. It passes the test if the suspension contains not less than 90 percent of the number of units of nystatin that it is represented to contain.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 39 FR 43833, Dec. 19, 1974; 49 FR 5098, Feb. 10, 1984; 50 FR 19920, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 449.104 Amphotericin B oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Amphotericin B oral suspension is a mixture of amphotericin B with one or more suitable and harmless preservatives, colorings, sweetening ingredients, flavorings, buffer substances, lubricants, suspending agents, and sequestrants in an aqueous vehicle. Each milliliter contains 100 milligrams of amphotericin B. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0. The amphotericin B conforms to the standards prescribed by § 449.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, loss on drying, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion into a high-speed glass blender with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot of the stock solution with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Further dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 449.120 Griseofulvin oral dosage forms.

§ 449.120a Griseofulvin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Griseofulvin tablets are tablets composed of griseofulvin, with or without one or more suitable fillers, colorings, lubricants, and binders. Each tablet contains 125, 250, or 500 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 5.0 percent. The tablets shall disintegrate within 1 hour. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch for griseofulvin content, loss on drying, and disintegration time.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Accurately weigh 20 tablets

and determine the average tablet weight. Grind the tablets to a fine powder in a mortar and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform and mix. Allow to settle and transfer 2.0 milliliters of the supernate to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per tablet as follows:

$$\text{Milligrams of griseofulvin per tablet} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

f=Potency of the griseofulvin working standard in micrograms per milligram;

W_a=Average tablet weight in milligrams;

W_s=Weight of the griseofulvin working standard in milligrams;

W_u=Weight of the ground tablet powder sample in milligrams;

V_u=Volume of the dissolved ground tablet powder sample in milliliters.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20660, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.120b Griseofulvin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin capsules are gelatin capsules containing griseofulvin with a suitable filler and binder, with or without a suitable lubricant. Each capsule contains 125 or 250 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 1.0 percent. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content and loss on drying.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Empty the contents of 20 capsules into a tared weighing bottle. Weigh the powder and calculate the average capsule weight. Mix the powder and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform and mix. Allow to settle and transfer 2.0 milliliters of the supernate to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per capsule as follows:

$$\text{Milligrams of griseofulvin per capsule} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram;

W_a=Average capsule fill weight in milligrams;

W_u=Weight of the ground tablet powder sample in milligrams;

V_u=Volume of the dissolved capsule powder sample in milliliters.

(2) *Loss of drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 43 FR 9800, Mar. 10, 1978; 44 FR 20661, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.120c Griseofulvin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin oral suspension is griseofulvin oral suspension with one or more suitable flavorings, colorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains 25 milligrams of griseofulvin. Its griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. Its pH is not less than 6.5 and not more than 7.5. The griseofulvin used conforms to the standards prescribed by § 449.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for griseofulvin content, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area, and crystallinity.

(*b*) The batch for griseofulvin content and pH.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Transfer an accurately measured portion of the oral suspension equivalent to 100 milligrams of griseofulvin into a 50-milliliter round-bottomed glass-stoppered centrifuge tube. Add 5 milliliters of water and 20 milliliters of a solvent mixture of ethyl acetate and chloroform (85:15). Shake the tube for 1 minute and centrifuge it briefly to separate the layers. Transfer most of the upper layer to a 100-milliliter volumetric flask being careful not to remove any of the lower aqueous layer. Repeat the extraction step with two additional 20-milliliter portions of the solvent mixture combining the extracts in the volumetric flask with the first 20-milliliter extract. Dilute to volume with the solvent mixture and mix. Place 2.0 milliliters of this solution in a conical centrifuge tube and evaporate the contents to dryness on a steam bath under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per milliliter as follows:

$$\text{Milligrams of griseofulvin per milliliter} = \frac{R_u \times W_s \times f \times 2}{R_s \times 1{,}000 \times V_o}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;

R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;

W_s=Weight of the griseofulvin working standard in milligrams;

f=Potency of the griseofulvin working standard in micrograms per milligram;
V_o=Volume of oral suspension taken in milliliters.

(2) *pH.* Proceed as directed in § 436.202 of this subchapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 44 FR 20662, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 449.120d Griseofulvin (ultramicrosize) tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Griseofulvin (ultramicrosize) tablets are composed of ultramicrosize crystals of griseofulvin which may or may not be dispersed in polyethylene glycol 6,000. Each tablet contains 125, 165, 250, or 330 milligrams of griseofulvin. The griseofulvin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of griseofulvin that it is represented to contain. The loss on drying is not more than 5.0 percent. It passes the solubility characteristic test. If it is dispersed in polyethylene glycol 6,000, the griseofulvin used conforms to the standards prescribed by § 449.20(a)(1). If it is not dispersed in polyethylene glycol 6,000, the griseofulvin used conforms to the standards prescribed by § 449.20(a)(1), except specific surface area.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for potency, loss on drying, melting point, specific rotation, identity, residue on ignition, heavy metals, specific surface area (if it is dispersed in polyethylene glycol 6,000), and crystallinity.

(*b*) The batch for griseofulvin content, loss on drying, and solubility characteristic.

(ii) Samples required:

(*a*) The griseofulvin used in making the batch: 10 packages, each containing not less than 1 gram.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Griseofulvin content (gas liquid chromatography).* Proceed as directed in § 436.321 of this chapter, except:

(i) Prepare the sample solution as follows: Accurately weigh 20 tablets and determine the average tablet weight. Grind the tablets to a fine powder in a mortar and transfer an accurately weighed sample to a volumetric flask of such size that for each 50 milliliters of volume there are 40 milligrams of griseofulvin (estimated). Add chloroform to about one-fourth volume of the flask. Swirl the flask and apply gentle heat to aid in dissolution of the griseofulvin. Allow the mixture to cool and then dilute to volume with chloroform. Mix and allow to settle. Using gentle vacuum, remove and discard the waxy substance that forms on the top of the chloroform. Transfer 2.0 milliliters of the chloroform solution to a conical centrifuge tube and evaporate to dryness under a current of dry air. Add 1.0 milliliter of the internal standard solution to the centrifuge tube and mix vigorously to obtain a uniform solution; and,

(ii) Calculate the milligrams of griseofulvin per tablet as follows:

$$\text{Milligrams of griseofulvin per tablet} = \frac{R_u \times W_s \times f \times W_a \times V_u}{R_s \times W_u \times 1{,}000 \times 50}$$

where:

R_u=Area of the griseofulvin sample peak (at a retention time equal to that observed for the griseofulvin standard)/Area of the internal standard peak;
R_s=Area of the griseofulvin working standard peak/Area of the internal standard peak;
W_s=Weight of the griseofulvin working standard in milligrams;
f=Potency of the griseofulvin working standard in micrograms per milligram;
W_a=Average tablet weight in milligrams;
W_u=Weight of the ground tablet powder sample in milligrams;
V_u=Volume of the dissolved ground tablet powder sample in milliliters.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Solubility characteristic test.* Proceed as directed in § 436.317 of this chapter.

[40 FR 41523, Sept. 8, 1975, as amended at 43 FR 22676, May 26, 1978; 46 FR 7275, Jan. 23, 1981; 46 FR 21361, Apr. 10, 1981; 46 FR 46313, Sept. 18, 1981; 47 FR 34132, Aug. 6, 1982; 50 FR 19920, May 13, 1985]

§ 449.150 Nystatin oral dosage forms.

§ 449.150a Nystatin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin tablets are tablets composed of nystatin and suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 500,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The loss on drying is not more than 8 percent. The tablets shall disintegrate within 2 hours. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985; 50 FR 52772, Dec. 26, 1985]

§ 449.150b Nystatin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin oral suspension is a suspension containing nystatin and one or more suitable preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.0; except, if the product contains glycerin, its pH is not less than 6.0 and not more than 7.5. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative aliquot of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5

minutes. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Remove and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§449.150c Nystatin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin for oral suspension is a dry powder consisting of nystatin, and suitable and harmless suspending substances, preservatives, diluents, colorings, and flavorings. When the suspension is prepared as directed in its labeling, each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of aliquot of the stock solution and further the number of units of nystatin that it is represented to contain. The pH of the reconstituted drug is not less than 4.9 and not more than 5.5. Its moisture content is not more than 7.0 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, moisture and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Blend an appropriate aliquot in a high-speed glass blender for 3 to 5 minutes, using sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter. Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Using the dry powder, proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the suspension after reconstituting as directed in the labeling.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§449.150d Nystatin pastilles.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin pastilles are composed of nystatin with suitable diluents, binders, buffers, colorings, and flavorings. Each pastille contains nystatin equivalent to 200,000 units of nystatin. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of nystatin that it is represented to contain. The pH in an aqueous solution is not less than 5.0 and not more than 7.5. It disintegrates within 90 minutes. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, pH, and disintegration time.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 pastilles.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of pastilles into a high-speed glass blender jar containing 100 milliliters of sterile distilled water. Blend for 18 to 20 minutes. Add 400 milliliters of dimethylformamide and continue blending for an additional 10 minutes. Remove an aliquot and add sufficient 80 percent dimethylformamide so that upon final dilution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter, the concentration of dimethylformamide will be 4 percent.

(2) *pH*. Dissolve 1 pastille in 100 milliliters of distilled water at 37 °C, cool, and proceed as directed in § 436.202 of this chapter.

(3) *Disintegration time*. Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(4) of that section.

[52 FR 4617, Feb. 13, 1987; 52 FR 7741, Mar. 12, 1987, as amended at 55 FR 11584, Mar. 29, 1990]

Subpart C—Injectable Dosage Forms

§ 449.204 Amphotericin B for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Amphotericin B for injection is a dry mixture containing in each immediate container 50 milligrams of amphotericin B, 41 milligrams of sodium desoxycholate, and suitable buffering substances. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of amphotericin B that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 8.0 percent. Its pH in an aqueous solution containing 10 milligrams of amphotericin B per milliliter is not less than 7.2 and not more than 8.0. The amphotericin B used conforms to the standards prescribed by § 449.4a(a)(1).

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5 of this chapter, each package shall bear on its label and labeling the following statement: ''For intravenous infusion in hospitals only''.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, loss on drying, pH, residue on ignition, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable syringe and hypodermic needle, remove all of the withdrawable contents if the container is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot of this solution and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(e) of this chapter, using a solution containing 2 milligrams of amphotericin B per milliliter, except in lieu of paragraph (a)(3), if no rabbit shows an individual rise in temperature of 1.1° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 3° C., the sample meets the requirements for absence of pyrogen. If one or two rabbits show a temperature rise of 1.1° C. or more, or if the sum of temperature rises exceeds 3° C., repeat the test using five other rabbits. If not more than three of the eight rabbits show a temperature rise of 1.1° C. or more, and if the sum of the temperature rises does not exceed 8° C. the sample meets the requirements for absence of pyrogens.

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter using an aqueous solution containing 10 milligrams of amphotericin B per milliliter.

[39 FR 19134, May 30, 1974, as amended at 45 FR 16472, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

Subpart D—Ophthalmic Dosage Forms

§ 449.340 Natamycin ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Natamycin ophthalmic suspension contains natamycin with one or more suitable and harmless preservatives in a suitable and harmless aqueous vehicle. Each milliliter contains 50 milligrams of natamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of natamycin that it is represented to contain. It is sterile. Its pH is not less than 5.0 and not more than 7.5. The natamycin used conforms to the standards prescribed by § 449.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The natamycin used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The natamycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Dilute solutions of natamycin are very sensitive to light and should be kept in the dark as much as possible or substantial decomposition will take place.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with dimethylsulfoxide to a concentration of 100 micrograms of natamycin per milliliter (estimated). Further dilute an aliquot with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 5.0 micrograms of natamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of sample in lieu of 1.0 milliliter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[43 FR 55384, Nov. 28, 1978, as amended at 48 FR 51293, Nov. 8, 1983]

Subpart E [Reserved]

Subpart F—Dermatologic Dosage Forms

§ 449.504 Amphotericin B dermatologic dosage forms.

§ 449.504a Amphotericin B ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B ointment is composed of amphotericin B in a suitable and harmless ointment base. It may contain suitable and harmless coloring agents and protectants. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B that it is represented to contain. Its moisture content is not more than 1.0 percent. The amphotericin B used conforms to the standards prescribed by § 449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample (usually 1 gram) into an appropriate-sized Erlenmeyer flask with 10 milliliters of ethyl ether. Allow to dissolve for 1 hour with the intermittent manual shaking. Add a measured amount of dimethylsulfoxide to the flask and place on a shaker for 10 minutes. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.504b Amphotericin B cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B cream is composed of amphotericin B, with or without one or more suitable and harmless emollients, perfumes, dispersants, and preservatives, in a suitable and harmless cream base. It contains 30 milligrams of amphotericin B in each gram. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per gram that it is represented to contain. The amphotericin B used conforms to the standards prescribed by § 449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: With the aid of a high-speed glass blender, dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to a concentration of 20 micrograms of

amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

§449.504c Amphotericin B lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B lotion is composed of amphotericin B in a suitable and harmless lotion vehicle. It contains suitable and harmless emollients, emulsifiers, coloring agents, diluents, preservatives, and perfumes. It contains 30 milligrams of amphotericin B per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of amphotericin B per milliliter that it is represented to contain. Its pH is not less than 5.0 and not more than 7.0. The amphotericin B used conforms to the standards prescribed by §449.4(a)(1) (i), (ii), (v), (vi), and (vii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, pH, residue on ignition, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The amphotericin B used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an aliquot in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute the stock solution with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot and dilute with 0.2*M* potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted lotion.

§449.550 Nystatin dermatologic dosage forms.

§449.550a Nystatin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin ointment is composed of nystatin and a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by §449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

§ 449.550b Nystatin-iodochlorhydroxyquin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-iodochlorhydroxyquin ointment is composed of nystatin and iodochlorhydroxyquin in a suitable and harmless ointment base. Each gram contains 100,000 units of nystatin and 10 milligrams of iodochlorhydroxyquin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its iodochlorhydroxyquin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of iodochlorhydroxyquin that it is represented to contain. It passes the identity test for iodochlorhydroxyquin. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1). The iodochlorhydroxyquin used conforms to the standards prescribed by U.S.P. XVIII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The iodochlorhydroxyquin used in making the batch for all U.S.P. XVIII specifications.

(*c*) The batch for nystatin content, iodochlorhydroxyquin content, iodochlorhydroxyquin identity, and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Iodochlorhydroxyquin content*—(i) *Reagents.* (*a*) Ferric chloride reagent. Dissolve 1.0 gram of ferric chloride ($FeCl_3 \cdot 6H_2O$) in a mixture of 1.0 milliliter of concentrated hydrochloric acid and sufficient distilled water to make 1 liter.

(*b*) Acetone, reagent grade.

(*c*) 2-Methoxyethanol, reagent grade.

(ii) *Preparation of standard solution.* Dissolve an accurately weighed portion of iodochlorhydroxyquin U.S.P. reference standard in sufficient 2-methoxyethanol to make a solution containing 1.0 milligram of iodochlorhydroxyquin per milliliter. Transfer 5.0 milliliters of this standard solution to a 50-milliliter volumetric flask.

(iii) *Preparation of sample solution.* Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly. Transfer a 10-milliliter aliquot of the acetone solution to a 50-milliliter volumetric flask and evaporate on a steam bath. To the residue, add 20 milliliters of 2-methoxyethanol and swirl to dissolve the iodochlorhydroxyquin.

(iv) *Procedure.* To each flask containing standard solution and sample solution, respectively, add 2.0 milliliters of ferric chloride reagent and dilute to

volume with 2-methoxyethanol. Mix thoroughly. Using a suitable spectrophotometer equipped with 1.0-centimeter cells and a blank prepared by diluting 2.0 milliliters of ferric chloride reagent to 50 milliliters with 2-methoxyethanol, determine the absorbance of the sample and standard solutions at 650 nanometers. Set the instrument to 100-percent transmission with the blank.

(v) *Calculation.*

Milligrams of iodochlorhydroxyquin per gram of sample=(Absorbance of sample × 50)/(Absorbance of standard × Weight of sample in grams)

(3) *Iodochlorhydroxyquin identity.* Proceed as directed in § 436.400 of this chapter, preparing the sample solution as follows: Accurately weigh a portion of the sample equivalent to 50 milligrams of iodochlorhydroxyquin into a 125-milliliter Erlenmeyer flask. Add 50 milliliters of acetone, warm on a steam bath, and shake gently. Cool to room temperature and filter contents through a pledget of glass wool into a 100-milliliter volumetric flask. Wash the Erlenmeyer flask with two 20-milliliter portions of acetone and filter the washings into the volumetric flask. Dilute to volume with acetone and mix thoroughly.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 449.550c Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide ointment; nystatin-neomycin sulfate-gramicidin-fludrocortisone acetate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* The drug is nystatin, neomycin sulfate, gramicidin, and either triamcinolone acetonide or fludrocortisone acetate in a suitable ointment base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and either 1.0 milligram of triamcinolone acetonide or 1.0 milligram of fludrocortisone acetate. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its moisture content is not more than 0.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes with sufficient dimethylformamide to give a

concentration of 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogenous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh and dissolve a representative portion of the sample in approximately 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent alcohol prepared from alcohol U.S.P. XX. Repeat the extraction three times. Combine the extractives in a suitable volumetric flask, bring to volume with alcohol U.S.P. XX, and mix well. Further dilute with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 449.550d Nystatin cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin cream is composed of nystatin and suitable and harmless emulsifiers, perfumes, buffers, preservatives, and a protectant in a suitable and harmless cream base. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give an estimated concentration of 400 units of nystatin per milliliter, blend an accurately weighed representative portion in a high-speed blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

§ 449.550e Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin-triamcinolone acetonide cream is composed of nystatin, neomycin sulfate, gramicidin, triamcinolone acetonide, and suitable and harmless emulsifiers, solvents, perfumes, buffers, preservatives, and a protectant in a suitable cream base. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1 milligram of

triamcinolone acetonide. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. The nystatin used conforms to the standards prescribed by §449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by §444.42(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by §448.25(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for nystatin content, neomycin content, and gramicidin content.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Using sufficient dimethylformamide to give a concentration of 400 units of nystatin (estimated) per milliliter, blend an accurately weighed representative portion in a high-speed glass blender for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the cream in a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and either until homogeneous. Add 20 to 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to ensure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Accurately weigh a representative portion of the sample and dissolve in approximately 50 milliliters of petroleum ether in a separatory funnel. Extract with 20 milliliters of 80 percent alcohol prepared from alcohol U.S.P. XX. Repeat the extraction three times. Combine the extractives in a suitable volumetric flask, bring to volume with alcohol U.S.P. XX, and mix well. Further dilute with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

[39 FR 19134, May 30, 1974, as amended at 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§449.550f Nystatin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Nystatin topical powder is a dry powder composed of nystatin and talc. Each gram contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

§ 449.550g Nystatin-neomycin sulfate-gramicidin topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin topical powder is a dry powder composed of nystatin, neomycin sulfate, gramicidin, and talc. Each gram contains 100,000 units of nystatin, 2.5 milligrams of neomycin and 0.25 milligram of gramicidin. Its nystatin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its gramicidin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of gramicidin that it is represented to contain. Its loss on drying is not more than 2.0 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the standards prescribed by § 444.42a(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by § 448.25(a)(1) (i), (iii), (iv), (v) and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, loss on drying, residue on ignition, melting point, crystallinity, and identity.

(*d*) The batch for nystatin content, neomycin content, gramicidin content, and loss on drying.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages each consisting of 300 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 300 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of seven immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend the entire contents of an accurately weighed representative portion of the sample for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend an accurately weighed representative sample for 3 to 5 minutes in sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a convenient concentration. Further dilute an aliquot with solution 3 to the reference concentration of 1 microgram of neomycin per milliliter (estimated).

(iii) *Gramicidin content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed representative sample in alcohol U.S.P. XX and filter. Collect the filtrate and dilute a portion with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[39 FR 19134, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§449.550h Nystatin lotion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin lotion is composed of nystatin with one or more suitable and harmless suspending agents, emulsifiers, surfactants, and preservatives in a suitable and harmless vehicle. Each milliliter contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. Its pH is not less than 5.5 and not more than 7.5. The nystatin used conforms to the standards prescribed by §449.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and dilute with sufficient dimethylformamide to yield a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[40 FR 3766, Jan. 24, 1975, as amended at 50 FR 19920, May 13, 1985]

Subpart G—Vaginal Dosage Forms

§449.610 Candicidin vaginal dosage forms.

§449.610a Candicidin vaginal ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal ointment is composed of candicidin and a suitable ointment base. It contains 0.6 milligram of candicidin per gram. Its

potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of candicidin that it is represented to contain. Its moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of § 449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Shake the sample and *n*- hexane until homogeneous. Add 15 milliliters of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole) and shake well. Allow the layers to separate. Remove the bottom layer and repeat the extraction procedure with a second 15-milliliter portion of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole). Combine the extractives in a suitable volumetric flask and fill to volume with sterile distilled water. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975]

§ 449.610b Candicidin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal tablets are tablets composed of candicidin with suitable binders, diluents, and lubricants. Each tablet contains 3 milligrams of candicidin. Its potency is satisfactory if it is not less than 90 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain, except that for the issuance of a certificate for each batch, the candicidin content must be not less than 115 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The tablets shall disintegrate within 30 minutes. The loss on drying is not more than 1 percent. The candicidin used in making the batch conforms to the standards of § 449.10(a)(1).

(2) *Labeling.* The drug shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) *Samples required.* (*a*) The candicidin used in making the batch: 10 packages, each consisting of approximately 300 milligrams.

(*b*) The batch: A minimum of 56 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Weigh a pool of five tablets and grind in a mortar to a very fine powder. Suspend an accurately weighed aliquot (of approximately 2 grams) in 10 milliliters of dimethylsulfoxide. Centrifuge for 5 minutes at 2,000 revolutions per minute. Carefully decant the supernatant solution into a sterile 250-milliliter volumetric flask. Wash the residue three times with 5-milliliter portions of dimethylsulfoxide, centrifuging each time. Add the washes to the 250-milliliter volumetric flask

and fill to volume with sterile distilled water. Using sterile distilled water, further dilute to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the method described in paragraph (e)(1) of that section, except use distilled water as the immersion fluid.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

§449.610c Candicidin vaginal capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Candicidin vaginal capsules are gelatin capsules containing 3 milligrams of candicidin in a suitable and harmless ointment. The candicidin content is satisfactory if it is not less than 90 percent and not more than 150 percent of the number of milligrams of candicidin that it is represented to contain. The moisture content is not more than 0.1 percent. The candicidin used conforms to the requirements of §449.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The candicidin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The candicidin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 20 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Remove the tips from two capsules and express the ointment from each capsule into a separatory funnel containing approximately 50 milliliters of *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Wash out the capsules at least two times with 2- to 3-milliliter portions of warm (approximately 50° C) *n*- hexane (containing 0.1 percent butylated hydroxyanisole). Add the washes to the separatory funnel. Shake the sample and *n*- hexane until homogeneous. Add 15 milliliters of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole) and shake well. Allow the layers to separate. Remove the bottom layer and repeat the extraction procedure with a second 15-milliliter portion of dimethylsulfoxide (containing 0.1 percent butylated hydroxyanisole). Combine the extractives in a suitable volumetric flask and fill to volume with sterile distilled water. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of candicidin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19134, May 30, 1974, as amended at 40 FR 15089, Apr. 4, 1975]

§449.650 Nystatin vaginal dosage forms.

§449.650a Nystatin vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin vaginal tablets are tablets composed of nystatin and suitable and harmless diluents, binders, and lubricants. Each tablet contains 100,000 units of nystatin. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of nystatin that it is represented to contain. The loss on drying is not more than 5 percent. The disintegration time is not more than 1 hour. The nystatin used conforms to the standards prescribed therefor by §449.50(a)(1) (i), (iii), (iv), and (v).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for nystatin content, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 immediate containers of approximately 300 milligrams each.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time*. Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section, except use distilled water in lieu of gastric fluid.

[39 FR 19134, May 30, 1974. Redesignated at 43 FR 43458, Sept. 26, 1978]

§ 449.650b Nystatin vaginal suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Nystatin vaginal suppositories contain in each suppository 100,000 units of nystatin in a suitable and harmless water soluble base. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its moisture content is not more than 1.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 suppositories.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of suppositories into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot with sufficient dimethylformamide to obtain a concentration of 400 units of nystatin per milliliter (estimated). Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

[43 FR 43458, Sept. 26, 1978, as amended at 50 FR 19920, May 13, 1985]

PART 450—ANTITUMOR ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
450.10a Sterile bleomycin sulfate.
450.20 Dactinomycin.
450.22 Daunorubicin hydrochloride.
450.24 Doxorubicin hydrochloride.
450.30 Idarubicin hydrochloride.
450.40 Plicamycin.
450.45 Mitomycin.

Subpart B [Reserved]

Subpart C—Injectable Dosage Forms

450.210 Sterile bleomycin sulfate.
450.220 Dactinomycin for injection.
450.222 Daunorubicin hydrochloride for injection.
450.224 Doxorubicin hydrochloride injectable dosage forms.
450.224a Doxorubicin hydrochloride for injection.
450.224b Doxorubicin hydrochloride injection.
450.230 Idarubicin hydrochloride for injection.
450.240 Plicamycin for injection.
450.245 Mitomycin for injection.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§450.10a Sterile bleomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile bleomycin sulfate is the amorphous sulfate salt of bleomycin. Bleomycin has been separated into several similar glyco-peptide molecules. It is a cream-colored powder that is so purified and dried that:

(i) Its potency is not less than 1.5 units and not more than 2.0 units of bleomycin per milligram. If it is packaged for dispensing, the content of the ampoule or vial is not less than 90 percent and not more than 120 percent of the number of units of bleomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 6.0 percent.

(vii) Its pH in an aqueous solution containing 10 units per milliliter is not less than 4.5 and not more than 6.0.

(viii) Its copper content is not greater than 0.1 percent.

(ix) Its content of various bleomycins is as follows: Bleomycin A_2 is not less than 55 percent and not more than 70 percent; bleomycin B_2 is not less than 25 percent and not more than 32 percent; bleomycin B_4 is not more than 1 percent. Bleomycins A_2 and B_2 should comprise not less than 85 percent of the total bleomycins.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, copper, content of various bleomycins, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: A minimum of 20 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 7.0 (solution 16), to provide a stock solution of convenient concentration; if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents. Dilute the sample thus obtained with solution 16 to provide a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 16 to the reference concentration of 0.04 unit of activity per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 0.5 unit of bleomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in §436.200(a) of this chapter, using the total contents of 2 or 3 vials.

(7) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 units per milliliter.

(8) *Copper content*—(i) *Reagents.* Dissolve 10 milligrams of zinc dibenzyldithiocarbamate in 100 milliliters of carbon tetrachloride.

(ii) *Preparation of standard copper solution.* Accurately weigh 1.965 grams of cupric sulfate pentahydrate and transfer to a 1-liter volumetric flask. Dissolve the material in 0.1*N* hydrochloric acid, dilute to volume with 0.1*N* hydrochloric acid and mix well. Transfer 3 milliliters of this stock solution to a 1-liter volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix well. This standard copper solution contains 0.0015 milligram of copper per

milliliter. Transfer 10 milliliters of the standard copper solution to a 60-milliliter separatory funnel.

(iii) *Preparation of the sample.* Accurately weigh approximately 15 milligrams of sample into a 60-milliliter separatory funnel. Dissolve the sample in 10 milliliters of 0.1*N* hydrochloric acid.

(iv) *Procedure.* To the separatory funnels containing the sample solution and standard copper solution, add 10 milliliters of the zinc dibenzyldithiocarbamate solution and shake the funnels vigorously for 1 minute. Allow the phases to separate. Filter the carbon tetrachloride phase (lower phase) through 1 gram of anhydrous sodium sulfate to remove excess water. Using a suitable spectrophotometer equipped with 1-centimeter cells, and carbon tetrachloride as a blank, measure the absorbance of the standard copper solution and the sample solution at 435 nanometers. Calculate the percent copper as follows:

$$\text{Percent copper} = \frac{\text{Absorbance of sample solution} \times 1.5}{\text{Absorbance of standard copper solution} \times \text{Sample weight in milligrams}}$$

(9) *Content of various bleomycin fractions.* Proceed as directed in § 436.339 of this chapter.

(10) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(1) of that section, using a 1 percent mixture.

[40 FR 52005, Nov. 7, 1975; 40 FR 53998, Nov. 20, 1975, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51913, Nov. 15, 1983; 50 FR 19920, May 13, 1985]

§ 450.20 Dactinomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin is a bright-red compound that is so purified and dried that:

(i) Its dactinomycin content is not less than 900 micrograms of dactinomycin per milligram, calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 15 percent.

(iii) Its absorptivity at 445 nanometers is not less than 0.95 and not more than 1.03 times that of the dactinomycin working standard at the same wavelength. Its absorbance at 240 nanometers is not less than 1.3 and not more than 1.5 times its absorbance at 445 nanometers.

(iv) It is crystalline.

(v) It passes the identity test for dactinomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter, and in addition each package shall bear on its label the statement ''Protect from light and excessive heat.''

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(ii) Samples required: 16 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content.* Proceed as directed in § 436.331 of this chapter, preparing the sample and calculating the dactinomycin content as follows:

(i) *Preparation of sample solution.* Accurately weigh a sufficient amount of the sample to obtain a solution containing approximately 0.25 milligram per milliliter of dactinomycin in mobile phase.

(ii) *Calculations.* Calculate the micrograms of dactinomycin per milligram of sample as follows:

$$\text{Micrograms of dactinomycin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u = Area of the dactinomycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the dactinomycin peak in the chromatogram of the dactinomycin working standard;

P_s = Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Absorptivity*—(i) *Procedure.* Accurately weigh approximately 15 milligrams of the sample ''as is'' and 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter. Transfer each weighing to separate 100-milliliter volumetric flasks. Dissolve the material and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters of each solution into separate 25-milliliter volumetric flasks, dilute to volume with spectrophotometric-grade methyl alcohol. Mix well. Using a suitable spectrophotometer and 1-centimeter absorption cells, determine the absorbance of the sample solution at the 240-nanometer and at the 445-nanometer absorption peaks (the exact position of the peaks should be determined for the particular instrument used). Determine the absorbance of the standard at the 445-nanometer absorption peak.

(ii) *Calculations.* Calculate the relative absorptivity and the ratio for the absorbances of the sample as follows:

$$\text{Relative absorptivity at 445 nanometers} = \frac{A_2 \times \text{milligrams of standard} \times \text{potency of the standard in micrograms per milligram}}{A_3 \times \text{milligrams of sample} \times (100 - M) \times 10}$$

$$\text{Ratio for the absorbances of the sample at 240 and 445 nanometers} = \frac{A_1}{A_2}$$

where:

A_1=Absorbance at 240 nanometers for the sample;

A_2=Absorbance at 445 nanometers for the sample;

A_3=Absorbance at 445 nanometers for the standard;

M=Percent moisture in the sample.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the dactinomycin working standard.

[49 FR 6092, Feb. 17, 1984, as amended at 49 FR 24018, June 11, 1984; 50 FR 19675, May 10, 1985]

§ 450.22 Daunorubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride is the monohydrochloride salt of (1*s*,3*s*)-3-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl-3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside. It is a red-orange, hygroscopic powder. It is so purified and dried that:

(i) Its potency is not less than 842 micrograms and not more than 1,030 micrograms of daunorubicin per milligram.

(ii) Its moisture content is not more than 3.0 percent.

(iii) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It is crystalline.

(v) It passes the identity test for daunorubicin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples required: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Daunorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood. Wear rubber gloves, protective gowns, head coverings, and protective eye goggles when handling dry powders. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, promptly wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite solution.

(1) *Potency.* Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography shall be conclusive.

(i) *High-pressure liquid chromatography.* Proceed as directed in § 436.322 of this chapter, except in lieu of the mobile phase and pH described in paragraph (b)(2) of that section, use a mixture of water: acetonitrile (62:38) adjusted to pH 2.2±0.2 with phosphoric acid. Prepare the sample and standard solutions and calculate the daunorubicin content as follows:

(*a*) *Preparation of sample and working standard solutions.* Accurately weigh approximately 25 milligrams of the sample and of the daunorubicin working standard and dissolve each in 25 milliliters of the internal standard solution prepared as directed in § 436.322(b)(3) of this chapter.

(*b*) *Calculations.* Calculate the daunorubicin content as follows:

$$\text{Micrograms of daunorubicin per milligram} = \frac{R_u \times W_s \times P \times 100}{R_s \times W_u \times (100 - M)}$$

where:

R_u=Area of the daunorubicin sample peak/Area of the internal standard peak;

R_s=Area of the daunorubicin standard peak/Area of the internal standard peak;

W_s=Weight of the daunorubicin working standard in milligrams;

W_u=Weight of the sample in milligrams;

M=Moisture content of the sample in percent;

P=Potency of the daunorubicin working standard in micrograms per milligram.

(ii) *Microbiological turbidimetric assay for daunorubicin*—(*a*) *Preparation of working standard stock solutions and standard response line concentrations.* Dissolve an accurately weighed portion of the working standard with sufficient 0.054*M* sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter. The working standard stock solution may be stored under refrigeration for 1 week. Further dilute an aliquot of the stock solution with solution 18 to obtain standard response line concentrations of 4, 8, and 16 micrograms of daunorubicin activity per milliliter. The 8-micrograms-per-milliliter concentration is the reference concentration of the assay.

(*b*) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample with sufficient 0.054*M* sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 18 to the reference concentration of 8 micrograms of daunorubicin activity per milliliter (estimated).

(c) *Procedure for assay.* Place 1.0 milliliter of each concentration of the standard response line and of the sample solution in each set of replicate tubes (as described in § 436.100(b)(1) of this chapter). Eighteen tubes are used for the three-point standard response line and six for each sample. To each tube, add 9 milliliters of medium 3 (as listed in § 436.102(b)(3) of this chapter), inoculated with 2 milliliters of a suspension of test organism I per liter of medium 3. The suspension of test organism I is prepared as described in § 436.103 of this chapter, except incubate the slants and Roux bottle for 16 to 18 hours at 37° C. Place the inoculated tubes immediately in a water bath at 37° C for approximately 3 hours.

The absorbance value for the growth control should be approximately 0.70–0.75 and the absorbance values for the 16 and 4 micrograms per milliliter standard doses should be approximately 0.25–0.35 and 0.55–0.65, respectively. An adjustment of the inoculum may be necessary in order to obtain absorbance values to these approximate levels in a 3-hour time period. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable spectrophotometer, at a wavelength of 530 nanometers. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of medium 3, solution 18, and formaldehyde used in the assay.

(d) *Estimation of potency.* Estimate the potency of the sample as follows: Using the three x values and the three corresponding y values, calculate Σx, Σx^2,$(\Sigma x)^2$, Σy and Σxy. Calculate b, the slope (regression coefficient), and a, the Y-intercept of the standard response line by the following equations:

$$b = \frac{n\sum xy - \left(\sum x\right)\left(\sum y\right)}{n\sum x^2 - \left(\sum x\right)^2}$$

$$a = \frac{\sum y - b\sum x}{n}$$

where:

n=Number of standard doses;

x=Logarithm of the concentration in micrograms per milliliter of each dose of the standard curve;

y=Mean response of the six absorbance values for each dose of the standard.

Calculate the concentration of the sample solution X corresponding to the observed mean response of the sample solution Y by the following equation:

$$x = \text{antilog}\,\frac{Y - a}{b}$$

where:

X=The concentration of the sample solution in micrograms per milliliter;

Y=The mean response of the six absorbance values for reference concentration sample solutions.

Calculate the potency of the daunorubicin sample as follows:

$$\text{Potency of daunorubicin sample in micrograms per milligram} = \frac{X \times F}{W}$$

where:

F=125, the appropriate dilution factor of the daunorubicin sample;

W=Weight of sample in milligrams.

The following example illustrates the mathematical calculations of the potency of a sample solution:

Standard doses (micrograms per milliliter)	16.0	8.0	4.0	n =	3
Log doses (x)	1.20412	0.90309	0.60206	Σx =	2.70927
				$(\Sigma x)^2$ =	7.34014
x^2	1.4499	0.81557	0.36248	Σx^2 =	2.62795
Absorbance readings	0.247	0.483	0.583	.....	
	0.236	0.414	0.584	.....	
	0.241	0.446	0.574	.....	
	0.236	0.423	0.555	.....	
	0.233	0.416	0.578	.....	
	0.243	0.413	0.559	.....	
Mean responses (y)	0.239	0.433	0.572	Σy =	1.244
xy	0.28778	0.39104	0.34438	Σxy =	1.0232

$$b = \frac{3(1.0232) - (2.70927)(1.244)}{3\ (2.62795 -)7.34014} = -0.553$$

$$a = \frac{1.244 - (-0.553)(2.70927)}{3} = 0.914$$

Mean response, Y, of sample solution=0.405.

$$\text{Calculated concentration, X, sample solution} = \text{antilog}\frac{0.405-0.914}{-0.553} = 8.32 \text{ micrograms per milliliter}$$

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1)(i) of this section compares qualitatively to that of the daunorubicin working standard.

[45 FR 75195, Nov. 14, 1980]

§ 450.24 Doxorubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxorubicin hydrochloride is the monohydrochloride salt of (8*S*, 10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*- hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione. It is a red-orange, almost completely odorless, hygroscopic powder. It is so purified and dried that:

(i) Its doxorubicin hydrochloride content is not less than 970 micrograms and not more than 1,020 micrograms of doxorubicin hydrochloride per milligram on the anhydrous and solvent free basis.

(ii) Its total solvent residue (as acetone and alcohol) is not more than 2.5 percent.

(iii) It contains no depressor substances.

(iv) Its moisture content is not more than 4.0 percent.

(v) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 4.0 and not more than 5.5.

(vi) It is crystalline.

(vii) It passes the identity test for doxorubicin.

(viii) The total of any impurities detected by high-pressure liquid chromatography assay is not more than 3.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each request shall contain:

(i) Results of tests and assays on the batch for doxorubicin hydrochloride content, solvent residue, depressor substances, moisture, pH, crystallinity, identity, and total impurities.

(ii) Samples required: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4.6-millimeter X 25-centimeter column packed with microparticulate (5 to 10 micrometers in diameter) packing material, such as trimethylsilane chemically bonded to porous silica, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Prepare a suitable mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 gram of sodium lauryl sulfate in 1,000 milliliters of this solution, adjust with 2*N* sodium hydroxide to a pH of 3.6±0.1. Filter through a suitable

filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution*. Dissolve an accurately weighed quantity of doxorubicin hydrochloride working standard in mobile phase to obtain a solution having a known concentration of 0.1 milligram of doxorubicin hydrochloride per milliliter.

(B) *Sample solution*. Transfer approximately 20 milligrams of sample, accurately weighed, to a 200-milliliter volumetric flask, add mobile phase to volume, and mix. This yields a solution containing 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(C) *Resolution test solution*. Use either of the following preparation methods:

(*1*) To 2 milliliters of a 1.0 milligram per milliliter solution of doxorubicin hydrochloride, add 20 microliters of 1*N* hydrochloric acid. Hold for 30 minutes at 95 °C in an oil bath.

(*2*) Dissolve about 10 milligrams of doxorubicin hydrochloride in 5 milliliters of water, add 5 milliliters of phosphoric acid, and allow to stand for about 30 minutes. Adjust with 2*N* sodium hydroxide (about 37 milliliters) to a pH of 2.6±0.1, add 15 milliliters of acetonitrile and 10 milliliters of methanol, mix, and filter. (Note: Portions of this solution may be frozen until needed, then thawed and mixed before use.)

(*3*) The procedures in paragraphs (b)(1)(ii)(C)(*1*) and (b)(1)(ii)(C)(*2*) of this section generate doxorubicinone, the aglycone of doxorubicin. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—(A) *Asymmetry factor*. The asymmetry factor (A_S (for the doxorubicin peak measured at a point 5 percent of the peak height is not less than 0.7 and not more than 1.2.

(B) *Efficiency of the column*. The absolute column efficiency (h_r (is satisfactory if it is not greater than 10.0, equivalent to 2,500 theoretical plates for a 25-centimeter column of 10-micrometer particles.

(C) *Resolution*. The resolution (*R*) between the peaks of doxorubicin and doxorubicinone (generated in situ) is satisfactory if it is not less than 5.5.

(D) *Capacity factor*. The capacity factor (*k*) for doxorubicin is satisfactory if it is in the range between 1.0 and 5.0.

(E) *Coefficient of variation*. The coefficient of variation (*relative standard of deviation* in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of doxorubicin hydrochloride per milligram of sample as follows:

$$\text{Micrograms of doxorubicin hydrochloride per milligram} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m - X)}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter;

C_U = Milligrams of the sample per milliliter of sample solution;

m = Percent moisture content of the sample; and

X = Percent solvent residue determined as directed in paragraph (b)(2) of this section.

(2) *Residue solvent (as acetone and alcohol)*—(i) *Standard preparation*. Transfer to a 100-milliliter volumetric flask about 200 milligrams of acetone, 300 milligrams of dehydrated alcohol, and 1,000 milligrams of dioxane, each accurately weighed, and mix. Dilute with water to volume, and mix. Transfer 5.0 milliliters of the resulting solution to a 50-milliliter volumetric flask, dilute with water to volume, and mix. This solution contains about 0.2 milligram of acetone, 0.3 milligram of alcohol, and 1 milligram of dioxane per milliliter.

(ii) *Solvent.* Transfer about 100 milligrams of dioxane, accurately weighed to a 100-milliliter volumetric flask, dilute with water to volume, and mix.

(iii) *Test preparation.* Dissolve about 200 milligrams of doxorubicin hydrochloride sample in 3.0 milliliters of solvent.

(iv) *Chromatographic system (see United States Pharmacopeia (U.S.P.) Chromatography (621)).* The gas chromatograph is equipped with a flame-ionization detector and a 4-millimeter X 2-meter column packed with 8-percent liquid phase G16 (see U.S.P. Chromatographic Reagents—Phases) on 100- to 120-mesh support S1AB (potassium hydroxide-washed) (see U.S.P. Chromatographic Reagents—Supports). The column is maintained at about 60 °C, and helium is used as the carrier gas. Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 minutes. Chromatograph the standard preparation, and record the peak responses as directed under procedure; the resolution (*R*) between adjacent peaks is not less than 2.0; the relative standard deviations of the ratios of the peak responses of the acetone and dioxane peaks and of the alcohol and dioxane peaks for replicate injections is not more than 4.0 percent; and the tailing factor for the alcohol peak is not more than 1.5.

(v) *Procedure.* (Note: Use peak areas where peak responses are indicated.) Separately inject equal volumes (about 1 microliter) of the standard preparation and the test preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.2 for acetone, 0.5 for alcohol, and 1.0 for dioxane. Calculate the percentage, by weight, of acetone and alcohol, respectively, in the sample as follows:

X = Percent acetone or alcohol = $100(C_A/C_D)(D_U/W_U)(R_U/R_S)$

where:

C_A = Concentration of acetone or alcohol in the standard preparation in milligrams per milliliter;

C_D = Concentration of dioxane in the standard preparation in milligrams per milliliter;

D_U = Total quantity of dioxane in the test preparation, in milligrams;

W_U = Quantity of doxorubicin hydrochloride taken to prepare the test preparation, in milligrams;

R_U = Response ratio of the analyte peak (acetone or alcohol) to the dioxane peak obtained from the test preparation; and

R_S = Response ratio of the analyte peak (acetoneoralcohol) to the dioxane peak obtained from the standard preparation.

The total of acetone and alcohol is not greater than 2.5 percent. Use the result obtained to calculate the doxorubicin hydrochloride content of the sample on the solvent-free basis.

(3) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the doxorubicin hydrochloride working standard.

(8) *Chromatographic purity.* Proceed as directed in paragraph (b)(1) of this section, except prepare the sample solution by dissolving the sample to be tested in mobile phase to obtain a solution containing approximately 0.5 milligram of doxorubicin hydrochloride per milliliter. Calculate the percentage of impurities as follows:

$$\text{Percent total impurities} = \frac{(100\,S)}{(S+r)}$$

where:

S = The sum of the responses of the minor component peaks; and

r = The response of the major doxorubicin hydrochloride peak.

The total related impurities detected is not more than 2.0 percent.

[41 FR 14184, Apr. 2, 1976; 41 FR 15844, Apr. 15, 1976, as amended at 42 FR 43063, Aug. 26, 1977; 43 FR 44836, Sept. 29, 1978; 47 FR 9396, Mar. 5, 1982; 47 FR 23710, June 1, 1982; 50 FR 19676, May 10, 1985; 53 FR 37292, Sept. 26, 1988; 59 FR 9639, Mar. 1, 1994]

§ 450.30 Idarubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Idarubicin hydrochloride is the monohydrochloride salt of 5,12-Naphthacenedione,9-acetyl-7-[(3-amino-2,3,6-trideoxy-α-*L-lyxo*-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-(7*S-cis*). It is an orange-red powder. It is so purified and dried that:

(i) Its idarubicin hydrochloride content is not less than 960 micrograms and not more than 1,030 micrograms of idarubicin hydrochloride per milligram on the anhydrous basis.

(ii) Its moisture content is not more than 5.0 percent.

(iii) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 6.5.

(iv) It is crystalline.

(v) The level of any individual impurity detected by high-performance liquid chromatography (HPLC) assay is not more than 1.0 percent.

(vi) The total of all detected impurities is not more than 3.0 percent.

(vii) It passes the identity test for idarubicin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for idarubicin hydrochloride content, solvent residues, moisture, pH, crystallinity, related individual thin-layer chromatography and HPLC impurities, total impurities, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay*. Idarubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders into a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Potency (HPLC)*. Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4.6-millimeter by 25-centimeter column packed with microparticulate (5 to 10 micrometers in diameter) packing material such as trimethylsilane chemically bonded to porous silica, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. The retention time for idarubicin hydrochloride is between 14 and 16 minutes. The retention time for the resolution compound 4-demethoxydaunorubicinone (generated in situ) is between 6 and 9 minutes. Mobile phase, diluent, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Prepare a suitably sized quantity of a mixture of water, acetonitrile, and methanol (540:290:170). Dissolve 1 gram of sodium lauryl sulfate and 2 milliliters of 85 percent phosphoric acid per liter of this solution. Adjust with 2 *N* sodium hydroxide to a pH of 3.6±0.1. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Diluent*. Prepare as mobile phase, excluding the sodium lauryl sulfate.

(iii) *Preparation of working standard solution*. Dissolve an accurately weighed quantity of idarubicin hydrochloride working standard in diluent to obtain a solution having a known concentration of 0.5 milligram of idarubicin hydrochloride per milliliter.

(iv) *Sample solution*. Transfer approximately 50 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask, add diluent to volume, and mix. This yields a solution containing 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(v) *Resolution test solution*. To 2 milliliters of a 1.0 milligram per milliliter aqueous solution of idarubicin hydrochloride, add 20 microliters of 1 *N* hydrochloric acid. Hold for 30 minutes at 95 C in an oil bath. This procedure generates the aglycone of idarubicin, 4-demethoxydaunorubicinone. Transfer 1.0 milliliter of this solution to a 10-milliliter volumetric flask, add diluent

to volume, and mix. Use this solution to determine the resolution requirement for the chromatographic system.

(vi) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_S), measure data point 5 percent of the peak height from the baseline, is satisfactory if it is not less than 0.85 and not more than 1.1.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 10.0 for the idarubicin hydrochloride peak, equivalent to 4,500 theoretical plates for a 25-centimeter column of 6-micrometer particles.

(C) *Resolution factor.* The resolution factor (R_S) between the peak for idarubicin and 4-demethoxydaunorubicinone (generated insitu) is satisfactory if it is not less than 9.5.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor.* The capacity factor (k') for idarubicin hydrochloride is satisfactory if it is not less than 5 and not more than 15. If the system suitability parameters have been met, proceed as described in § 436.216(b) of this chapter.

(vii) *Calculations.* Calculate the micrograms of idarubicin hydrochloride per milligram of sample as follows:

$$\text{Micrograms of idarubicin hydrochloride per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_U=Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_S=Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter;

C_U=Milligrams of idarubicin hydrochloride sample per milliliter of sample solution;

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *HPLC impurities.* Proceed as directed in paragraph (b)(1) of this section. Calculate the percentage of impurities as follows:

$$\text{Percent individual impurity} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC impurities} = \frac{A \times 100}{A_t}$$

where:

A_i=Area of the individual impurity peak;

A=The sum of areas of all peaks minus the area due to the idarubicin hydrochloride peak and solvent peak; and

A_t=The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as directed in § 436.211(b)(1).

[58 FR 26664, May 4, 1993]

§ 450.40 Plicamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Plicamycin is a yellow compound and is so purified and dried that:

(i) Its plicamycin content is not less than 900 micrograms of plicamycin per milligram calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 8 percent.

(iii) Its pH in an aqueous solution containing 0.5 milligram per milliliter is not less than 4.5 nor more than 5.5.

(iv) Its absorptivity on the anhydrous basis at the absorption maximum of 278 millimicrons is 100±5 percent of that of the plicamycin standard similarly treated.

(v) It gives a positive result to the identity tests for plicamycin.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label the statement ''Store below 10° C. (50° F.)''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for plicamycin content, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required on the batch: 2 packages, each containing not less than 100 milligrams; and 3 packages, each containing not less than 50 milligrams.

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Solutions should not be pipetted by mouth. Plicamycin is hygroscopic and care should be exercised during storage and weighing samples. Samples should be stored at 10° C. or less in a sealed, light-resistant container with a desiccant. Dispose of all waste material by dilution with larger volumes of trisodium phosphate solution.

(1) *Plicamycin content.* Proceed as directed in § 436.341 of this chapter, preparing the sample and calculating the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the micrograms of plicamycin per milligram of sample as follows:

$$\text{Micrograms of plicamycin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u = Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s = Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(g) of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 0.5 milligram of plicamycin per milliliter. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(4) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 10 milligrams each of the sample and standard (dried as described in § 436.200(g) of this chapter), accurately weighed, in 50 milliliters of absolute methanol. Transfer 5-milliliter portions into 100-milliliter volumetric flasks and dilute to volume with 0.01*N* hydrochloric acid in methanol prepared by diluting 20 milliliters of 0.5*N* aqueous hydrochloric acid to 1 liter with absolute methanol. Using a suitable spectrophotometer and 0.01*N* hydrochloric acid in methanol as the blank, scan the absorption spectrum between the wavelengths of 220 millimicrons and 400 millimicrons. Determine the absorbance of each solution at the absorption maximum near 278 millimicrons. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample solution} \times \text{milligrams of standard} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard solution} \times \text{milligrams of sample} \times 10}$$

(5) *Identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the plicamycin working standard.

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19145, May 30, 1974, as amended at 49 FR 5097, Feb. 10, 1984; 49 FR 24018, June 11, 1984]

§ 450.45 Mitomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Mitomycin is 7-amino-9a-methoxymitosane. It is a blue-violet compound that is soluble in water, methanol, acetone, butyl acetate, and cyclohexanone. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in a solution containing 5 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) When calculated on the anhydrous basis, its absorptivity at 357 nanometers is not less than 95 percent and not more than 105 percent of that of the mitomycin working standard similarly treated.

(vi) It gives a positive identity test for mitomycin.

(vii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: Five packages, each containing approximately 100 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of mitomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Absorptivity*. Determine the absorbance of the sample and standard solution in the following manner: Place an accurately weighed portion of approximately 25 milligrams of mitomycin into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute methanol. Further dilute an aliquot with absolute methanol to 0.005 milligram of mitomycin per milliliter. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and absolute methanol as the blank, determine the absorbance of the sample and standard solutions at 357 nanometers. Calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent mitomycin content of standard} \times 100}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where:

m=percent moisture in the sample.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19145, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

Subpart B [Reserved]

Subpart C—Injectable Dosage Forms

§ 450.210 Sterile bleomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile bleomycin sulfate packaged for dispensing are described in § 450.10a.

[40 FR 52006, Nov. 7, 1975]

§ 450.220 Dactinomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dactinomycin for injection is a dry mixture of dactinomycin and mannitol. Each container contains 0.5 milligram of dactinomycin. Its dactinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dactinomycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 4.0 percent. Its pH is not less than 5.5 and not more than 7.5. The dactinomycin used conforms to the standards prescribed by § 450.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter, and in addition each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container the statement ''Protect from light and excessive heat''.

(ii) On the outside wrapper or container and the immediate container the statement ''For hospitalized patients only''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The dactinomycin used in making the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(*b*) The batch for dactinomycin content, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The dactinomycin used in making the batch: 10 containers each containing not less than 40 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content.* Proceed as directed in § 436.331 of this chapter, except prepare the sample solution and calculate the dactinomycin content as follows:

(i) *Sample solution.* Reconstitute the vial with 2.0 milliliters of mobile phase. Shake well and filter if necessary.

(ii) *Calculations.* Calculate the dactinomycin content of the vial as follows:

$$\text{Milligrams of dactinomycin per vial} = \frac{A_u \times P_s \times d}{A_s \times 500}$$

where:

A_u=Area of the dactinomycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the dactinomycin peak in the chromatogram of the dactinomycin working standard;

P_s=Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, preparing the

sample for test as follows: Use a sufficient number of containers to yield 3 milligrams of dactinomycin. Reconstitute by adding 1.1 milliliters of sterile water for injection to each container. Aseptically pool the resultant solutions from each container. Dilute an accurately measured portion with sufficient diluent 1 to give a concentration of 0.2 milligram of dactinomycin per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

[39 FR 19145, May 30, 1974, as amended at 44 FR 10379, Feb. 20, 1979; 46 FR 16685, Mar. 13, 1981; 46 FR 46313, Sept. 18, 1981; 49 FR 6093, Feb. 17, 1984; 49 FR 15074, Apr. 17, 1984; 49 FR 24018, June 11, 1984; 50 FR 1504, Jan. 11, 1985; 50 FR 19676, May 10, 1985]

§ 450.222 Daunorubicin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride for injection is a freeze-dried powder whose components are daunorubicin hydrochloride and mannitol. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of daunorubicin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The daunorubicin hydrochloride used conforms to the standards prescribed by § 450.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The daunorubicin hydrochloride used in making the batch for potency, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, moisture, pH, and identity.

(ii) Samples required:

(*a*) The daunorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 34 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Daunorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood. Wear rubber gloves, protective gowns, head coverings, and protective eye goggles when handling dry powders. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with larger volumes of sodium hypochlorite solution.

(1) *Daunorubicin content (high-pressure liquid chromatography).* Proceed as directed in § 436.322 of this chapter, preparing the sample and standard solutions and calculating the daunorubicin content as follows:

(i) *Preparation of working standard solution.* Accurately weigh approximately 25 milligrams of the daunorubicin working standard and dissolve in 25 milliliters of the internal standard solution prepared as directed in § 436.322(b)(3) of this chapter.

(ii) *Preparation of sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate-sized volumetric flask with a sufficient amount of internal standard solution prepared as directed in § 436.322(b)(3) of this chapter, to obtain a concentration of 1.0 milligram of daunorubicin per milliliter.

(iii) *Calculations.* Calculate the daunorubicin content as follows:

$$\begin{array}{c}\text{Daunorubicin}\\ \text{content per vial}\\ \text{in milligrams}\end{array} = \frac{R_u \times W_s \times V \times P}{R_s \times 25 \times 1{,}000}$$

where:

$$R_u = \frac{\text{Area of the daunorubicin sample peak}}{\text{Area of the internal standard peak}}$$

$$R_s = \frac{\text{Area of the daunorubicin standard peak}}{\text{Area of the internal standard peak}}$$

W_s = Weight of the daunorubicin working standard in milligrams;
V = Volume in milliliters of the internal standard solution added to the vials;
P = Potency of the daunorubicin working standard in micrograms per milligram.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 2.25 milligrams of daunorubicin per milliliter.

(4) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in paragraph (d)(4) of that section.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling.

(7) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the daunorubicin working standard.

[45 FR 75198, Nov. 14, 1980, as amended at 50 FR 47214, Nov. 15, 1985]

§ 450.224 Doxorubicin hydrochloride injectable dosage forms.

§ 450.224a Doxorubicin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxorubicin hydrochloride for injection is a freeze-dried powder whose components are doxorubicin hydrochloride and lactose. It may also contain methylparaben. Its doxorubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of doxorubicin hydrochloride that it is represented to contain. It is sterile. It contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The doxorubicin hydrochloride used conforms to the standards prescribed by § 450.24(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxorubicin hydrochloride used in making the batch for doxorubicin hydrochloride content, residue solvents, depressor substances, moisture, pH, crystallinity, identity, and total related impurities.

(*b*) The batch for doxorubicin hydrochloride content, sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples required:

(*a*) The doxorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 34 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood while wearing rubber gloves. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography).* Proceed as directed in § 450.24(b)(1), preparing the sample solution and calculating the doxorubicin hydrochloride content as follows:

(i) *Sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate sized volumetric flask with sufficient mobile phase to obtain a concentration of 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the doxorubicin hydrochloride content per vial as follows:

$$\text{Milligrams of doxorubicin hydrochloride per vial} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia (U.S.P.) Bacterial Endotoxin Test, using a solution of doxorubicin hydrochloride for injection containing 1.1 milligrams of doxorubicin hydrochloride per milliliter. The specimen under test contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in paragraph (d)(4) of that section.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling, except in lieu of saline use distilled water.

(7) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the doxorubicin hydrochloride working standard.

[41 FR 14185, Apr. 2, 1976, as amended at 43 FR 44837, Sept. 29, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19676, May 10, 1985. Redesignated at 53 FR 37292, Sept. 26, 1988; 59 FR 9641, Mar. 1, 1994]

§ 450.224b Doxorubicin hydrochloride injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxorubicin hydrochloride injection is an aqueous solution of doxorubicin hydrochloride in an isosmotic diluent. Each milliliter contains doxorubicin hydrochloride equivalent to 2 milligrams of doxorubicin hydrochloride. Its doxorubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams it is represented to contain. It is sterile. It contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride. Its pH is not less than 2.5 and not more than 3.5. It passes the identity test. The doxorubicin hydrochloride used conforms to the standards prescribed by § 450.24(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The doxorubicin hydrochloride used in making the batch for doxorubicin hydrochloride content, residue solvents, depressor substances, moisture, pH, crystallinity, identity, and total related impurities.

(B) The batch for doxorubicin hydrochloride content, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples required:

(A) The doxorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 34 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood while wearing rubber gloves. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography).* Proceed as directed in § 450.24(b)(1), preparing the sample solution and calculating the doxorubicin hydrochloride content as follows:

(i) *Sample solution.* Dilute an accurately measured volume of sample equivalent to not less than 2 milligrams of doxorubicin hydrochloride, quantitatively with mobile phase to obtain a solution containing 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the milligrams of doxorubicin hydrochloride per milliliter of sample as follows:

$$\text{Milligrams of doxorubicin hydrochloride per milliliter} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) [Reserved]

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia (U.S.P.) Bacterial Endotoxin Test, using a test solution prepared by diluting doxorubicin hydrochloride injection with sterile water for injection to obtain a concentration of 1.1 milligrams of doxorubicin hydrochloride per milliliter. The specimen under test contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(6) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the doxorubicin hydrochloride working standard.

[53 FR 37292, Sept. 26, 1988, as amended at 59 FR 9641, Mar. 1, 1994]

§ 450.230 Idarubicin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Idarubicin hydrochloride for injection is a lyophilized mixture of idarubicin hydrochloride and lactose. Its idarubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of idarubicin hydrochloride that it is represented to contain. It is sterile. It contains not more than 8.93 U.S.P. endotoxin units per milligram of idarubicin hydrochloride. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.0. It passes the identity test. The idarubicin hydrochloride used conforms to the standards prescribed by § 450.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The idarubicin hydrochloride used in making the batch for idarubicin hydrochloride content, solvent residues, moisture, pH, crystallinity, related individual thin layer chromatography and high-performance liquid chromatography (HPLC) impurities, total impurities, and identity.

(B) The batch for idarubicin hydrochloride content, sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples required if requested by the Director, Center for Drug Evaluation and Research:

(A) The idarubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 34 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Idarubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders into a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Idarubicin hydrochloride content (HPLC).* Proceed as directed in § 450.30(b)(1), preparing the sample solution and calculating the idarubicin hydrochloride as follows:

(i) *Sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate-sized volumetric flask with sufficient diluent to obtain a concentration of 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the idarubicin hydrochloride content per vial as follows:

$$\text{Micrograms of plicamycin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_U=Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S=Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_S=Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(3) *Bacterial endotoxins.* Proceed as directed in the U.S.P. Bacteria endotoxin test. The specimen under test contains not more than 8.93 U.S.P. endotoxin units per milligram of idarubicin hydrochloride.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in § 436.201(d)(4).

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling, except use distilled water instead of saline.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the idarubicin hydrochloride working standard.

[58 FR 26665, May 4, 1993]

§ 450.240 Plicamycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Plicamycin for injection is a dry mixture of plicamycin and mannitol with or without a suitable buffer substance. Each immediate container contains 2.5 milligrams of plicamycin. Its plicamycin content is satisfactory if it contains not less than 90 percent and not more than 110 percent of the number of milligrams of plicamycin that it is representated to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. It contains no depressor substances. Its pH when reconstituted as directed in the labeling is not less than 5.0 and not more than 7.5. It passes the identity test for plicamycin. The plicamycin used conforms to the standards prescribed by § 450.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label or labeling the following as indicated:

(i) On the outside wrapper or container the statement ''Store below 10° C. (50° F.)''.

(ii) On the outside wrapper or container and on the immediate container the statement ''Mandatory: Before using read enclosed professional information carefully for dosage instructions and warnings''.

(iii) On the outside wrapper or container the statement ''Warning: For hospital use only. To be used under direct supervision of a physician''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The plicamycin used in making the batch for plicamycin content, loss on drying, absorptivity, pH, identity, and crystallinity.

(*b*) The batch for plicamycin content, sterility, pyrogens, moisture, Ph, depressor substances, and identity.

(ii) Samples required:

(*a*) The plicamycin used in making the batch: 3 packages, each containing not less than 50 milligrams; and 2 packages, each containing not less than 100 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 21 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Plicamycin is hygroscopic and care should be exercised during storage and weighing of samples. Dispose of all waste materials by dilution with larger volumes of trisodium phosphate solution. The samples should be stored at 10° C. or less in a sealed light-resistant container with a dessicant. Solutions should not be pipetted by mouth.

(1) *Plicamycin content.* Proceed as directed in § 436.341 of this chapter, except prepare the sample solution and calculate the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flash and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the plicamycin content of the vial as follows:

$$\text{Milligrams of plicamycin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s=Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Reconstitute the sample as directed in the labeling and proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 micrograms of plicamycin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the total contents of three to five vials.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(6) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(7) *Thin layer chromatography identity test for plicamycin*—(i) *Equipment*—(*a*) *Plates.* Use 20 by 20 centimeter or 15 by 20 centimeter thin layer chromatographic plates coated with Silica Gel Mixture, Chromatographic, U.S.P., to a thickness of 250 microns. Activate the plates by heating at 110° C. for 75 minutes. Place the plates in a desiccator until cooled to room temperature. Plates may be stored in a desiccator for 7 days.

(*b*) *Chamber* (*chromatographic*). A suitable chamber, equipped for thin layer chromatography.

(ii) *Preparations of solutions*—(*a*) *Solvent.* Mix reagent grade chloroform with reagent grade absolute methanol in volumetric proportions of 1:1.

(*b*) *Spray A.* Mix 50 milliliters of freshly prepared 1.0 percent ferric chloride in water (weight per volume), just before spraying, with 50 milliliters of freshly prepared 1.0 percent potassium ferricyanide in water (weight per volume).

(*c*) *Spray B.* Dissolve 2.28 grams of periodic acid in 100 milliliters of water. Dilute one volume of this periodic solution with 10 volumes of acetone.

(*d*) *Spray C.* Dissolve 184 milligrams of benzidine in a solution of 0.6 milliliter of acetic acid, 4.4 milliliters of water, and 95 milliliters of acetone.

(iii) *Preparation of spotting solutions*—(*a*) *Plicamycin standard solution.* Weigh 5 milligrams of plicamycin working standard and dissolve in 10 milliliters of methanol. Use the solution the same day it is prepared.

(*b*) *Plicamycin for injection sample solution.* Dilute with methanol to a concentration of 0.5 milligram of plicamycin per milliliter. Centrifuge and use the supernatant for spotting.

(*c*) *Mannitol reference solution.* Suspend 100 milligrams of mannitol in 5 milliliters of methanol. Centrifuge and use the supernatant for spotting.

(iv) *Procedure.* Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Spot duplicate plates as follows: On a line 2.5 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot 100 microliters (in four 25-microliter aliquots) of the standard solution, the sample solution, and the mannitol reference solution. Allow each aliquot to dry before applying subsequent volumes. After all spots are thoroughly dry, place the silica gel plates in the chromatographic chamber and develop by the ascending technique for approximately 60 minutes. Allow several minutes for the plates to air dry. On one plate, locate and record the position of fluorescent spots by examining under long wave ultraviolet light. Apply spray A and record the position of blue spots on the yellow-green background. On the other plate, locate the mannitol by first applying spray B, followed by spray C. The spots appearing white are mannitol. Measure the distance the solvent front traveled from the starting line and the distance the fluorescent spots are from the starting line. Calculate the R^f value by dividing the latter by the former. The plicamycin standard should have an R^f value of 0.7. If the standard has an R^f value greater than 0.8, the mobility of the standard may be decreased by increasing the ratio of the chloroform to methanol in the solvent to 3:2 or 3:1. Plicamycin appears as a single major component with the same R_f value as the plicamycin standard. It may show trace components at R_f values of about 0.5 and 0.4, and at the origin, which shall not be more intense than those shown by the plicamycin standard.

[39 FR 19145, May 30, 1974, as amended at 40 FR 1512, Jan. 8, 1975; 46 FR 60568, Dec. 11, 1981; 47 FR 9396, Mar. 5, 1982; 48 FR 11427, Mar. 18, 1983; 49 FR 5097, Feb. 10, 1984; 49 FR 24019, June 11, 1984; 50 FR 19676, May 10, 1985]

§ 450.245 Mitomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mitomycin for injection is a dry mixture of mitomycin and mannitol. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of mitomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its moisture content is not more than 5 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.0 and not more than 8.0. It passes the identity test for mitomycin. The mitomycin used conforms to the standards prescribed by § 450.45(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The mitomycin used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, moisture, pH, and identity.

(ii) Samples required:

(*a*) The mitomycin used in making the batch: Five packages, each containing approximately 100 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 25 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 1 microgram of mitomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 milligram of mitomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(6) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(7) *pH*. Proceed as directed in § 436.202 of this chapter using the drug reconstituted as directed in the labeling.

(8) *Identity*. Proceed as directed in § 436.310 of this chapter.

[39 FR 19145, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

PART 452—MACROLIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
452.10 Erythromycin.
452.15 Erythromycin estolate.
452.25 Erythromycin ethylsuccinate.
452.25a Sterile erythromycin ethylsuccinate.
452.30a Sterile erythromycin gluceptate.
452.32a Sterile erythromycin lactobionate.
452.35 Erythromycin stearate.
452.50 Clarithromycin.
452.60 Azithromycin.
452.75 Troleandomycin.

Subpart B—Oral Dosage Forms

452.110 Erythromycin oral dosage forms.
452.110a Erythromycin tablets.
452.110b Erythromycin enteric-coated tablets.
452.110c Erythromycin capsules.
452.110d Erythromycin particles in tablets.
452.115 Erythromycin estolate oral dosage forms.
452.115a Erythromycin estolate tablets.
452.115b Erythromycin estolate capsules.
452.115c Erythromycin estolate oral suspension.
452.115d Erythromycin estolate for oral suspension.
452.115e Erythromycin estolate for pediatric drops.
452.115f Erythromycin estolate chewable tablets.
452.115g Erythromycin estolate and sulfisoxazole acetyl oral suspension.
452.125 Erythromycin ethylsuccinate oral dosage forms.
452.125a Erythromycin ethylsuccinate chewable tablets.
452.125b Erythromycin ethylsuccinate oral suspension.
452.125c Erythromycin ethylsuccinate for oral suspension.
452.125d Erythromycin ethylsuccinate tablets.
452.125e Erythromycin ethylsuccinate-sulfisoxazole acetyl for oral suspension.
452.135 Erythromycin stearate oral dosage forms.
452.135a Erythromycin stearate tablets.
452.135b Erythromycin stearate oral suspension.
452.135c Erythromycin stearate for oral suspension.
452.150 Clarithromycin oral dosage forms.
452.150a Clarithromycin tablets.
452.150b Clarithromycin granules for oral suspension.

452.160 Azithromycin oral dosage forms.
452.160a Azithromycin capsules.
452.160b Azithromycin for oral suspension.
452.175 Troleandomycin oral dosage forms.
452.175a Troleandomycin capsules.
452.175b Troleandomycin oral suspension.
452.175c Troleandomycin for oral suspension.
452.175d Troleandomycin chewable tablets.

Subpart C—Injectable Dosage Forms

452.225 Erythromycin ethylsuccinate injection.
452.230 Sterile erythromycin gluceptate.
452.232 Erythromycin lactobionate injectable dosage forms.
452.232a Erythromycin lactobionate for injection.
452.232b Sterile erythromycin lactobionate.

Subpart D—Ophthalmic Dosage Forms

452.310 Erythromycin ophthalmic ointment.

Subpart E [Reserved]

Subpart F—Dermatologic Dosage Forms

452.510 Erythromycin dermatologic dosage forms.
452.510a Erythromycin ointment.
452.510b Erythromycin topical solution.
452.510d Erythromycin-benzoyl peroxide topical gel.
452.510e Erythromycin topical gel.

Subpart G [Reserved]

Subpart H—Rectal Dosage Forms

452.710 Erythromycin suppositories.

Subpart I [Reserved]

Subpart J—Certain Other Dosage Forms

452.910 Erythromycin for prescription compounding.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§ 452.10 Erythromycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin is the odorless, white to grayish-white or slightly yellow compound of a kind of erythromycin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It contains not less than 850 micrograms of erythromycin per milligram calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH is not less than 8.0 or more than 10.5.

(v) Its residue on ignition is not more than 2.0 percent.

(vi) Its heavy metals content is not more than 50 parts per million.

(vii) It gives a positive identity test for erythromycin.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, residue on ignition, heavy metals, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Dilute this solution further with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, except standardize the pH meter with pH 7.0 and pH 10.0 buffers and prepare the sample as follows: Dissolve 200 milligrams of sample in 5 milliliters of reagent grade methyl alcohol. Add 95 milliliters of water and mix. Record the pH when an equilibrium value has been reached.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

[39 FR 19149, May 30, 1974, as amended at 42 FR 38564, July 29, 1977; 43 FR 9801, Mar. 10, 1978; 50 FR 19920, May 13, 1985]

§ 452.15 Erythromycin estolate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate is the lauryl sulfate salt of the propionyl ester of a kind of erythromycin or a mixture of two or more such salts. It occurs as a white powder. It is soluble in alcohol, methyl alcohol, acetone, and chloroform, but is practically insoluble in water. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) Its free erythromycin content is not more than 3.0 percent.

(iii) Its moisture content is not more than 4.0 percent.

(iv) Its pH is not less than 4.5 nor more than 7.0.

(v) It gives positive identity tests for erythromycin estolate.

(vi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, free erythromycin content, moisture, pH, crystallinity, and identity.

(ii) Samples of the batch: A minimum of 10 containers, each containing not less than 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Immediately dilute this solution further with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 0.1 milligram of erythromycin per milliliter (estimated). Hydrolyze this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Free erythromycin content.* Proceed as directed in § 436.362 of this chapter.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Identity test.* Proceed as directed in § 436.211 of this chapter, preparing the sample as described in paragraph (b)(1) of that section.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, May 13, 1985; 53 FR 1920, Jan. 25, 1988]

§ 452.25 Erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate is the white, odorless, ethylsuccinate ester of erythromycin. It is so purified and dried that:

(i) It contains not less than 765 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 3.0 percent.

(iv) Its pH is not less than 6.0 and not more than 8.5.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives a positive identity test for erythromycin ethylsuccinate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a 1.0 percent suspension in water.

(5) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample prepared as described in paragraph (b)(3) of that section.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 452.25a Sterile erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin ethylsuccinate is the white, odorless, ethylsuccinate ester of erythromycin. It is so purified and dried that:

(i) It contains not less than 765 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) [Reserved]

(iv) Its moisture content is not more than 3.0 percent.

(v) Its pH is not less than 6.0 and not more than 8.5.

(vi) Its residue on ignition is not more than 1.0 percent.

(vii) It gives a positive identity test for erythromycin ethylsuccinate.

(viii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) [Reserved]

(4) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using a 1.0 percent suspension in water.

(6) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

§ 452.30a Sterile erythromycin gluceptate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin gluceptate is the white powder of the glucoheptonic

acid salt of erythromycin or a mixture of two or more such salts. It is freely soluble in water, alcohol, and methyl alcohol. It is slightly soluble in acetone and chloroform, but is practically insoluble in ether. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5.0 percent.

(vi) Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 6.0 nor more than 8.0.

(vii) It gives a positive identity test for erythromycin gluceptate.

(2) [Reserved]

(3) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing not less than 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers of the batch.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repacking or for use in manufacturing another drug, dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Dilute this solution further with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution containing 1.0 milligram of erythromycin base per milliliter (estimated). If it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with solution 3 to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in §436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using a concentration of 25 milligrams per milliliter.

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

[39 FR 19149, May 30, 1974, as amended at 46 FR 16685, Mar. 13, 1981; 50 FR 19920, 19921, May 13, 1985]

§452.32a Sterile erythromycin lactobionate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate is the white to off-white powder of the lactobionate salt of erythromycin or a mixture of two or more such salts. It is so purified and dried that:

(i) If the erythromycin lactobionate is not packaged for dispensing, its erythromycin potency is not less than 525 micrograms of erythromycin per milligram on an anhydrous basis. If the erythromycin lactobionate is packaged for dispensing, its erythromycin potency is not less than 525 micrograms of erythromycin per milligram on an anhydrous basis and also, each container contains not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 5.0 percent.

(v) Its pH is not less than 6.5 and not more than 7.5.

(vi) Its residue on ignition is not more than 2.0 percent.

(vii) Its heavy metals content is not more than 50 parts per million.

(viii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, residue on ignition, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 packages, each containing equal portions of approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows:

(i) *Product not packaged for dispensing (micrograms of erythromycin per milligram).* Dissolve an accurately weighed sample with sufficient methyl alcohol to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(ii) *Product packaged for dispensing.* Determine both micrograms of erythromycin per milligram of sample and milligrams of erythromycin per container. Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(ii)(*a*) and (*b*) of this section.

(*a*) *Micrograms of erythromycin per milligram.* Dissolve an accurately weighed sample with sufficient methyl alcohol to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(*b*) *Milligrams of erythromycin per container.* Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute an aliquot of the solution thus obtained with sterile distilled water to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 50 milligrams of erythromycin per milliliter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(8) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

[51 FR 35215, Oct. 2, 1986, as amended at 55 FR 11584, Mar. 29, 1990]

§ 452.35 Erythromycin stearate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate is the odorless, white or slightly yellow powder of the stearic acid salt of erythromycin. It is practically insoluble in water but is soluble in alcohol, methyl alcohol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 550 micrograms of erythromycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 4.0 percent.

(iv) Its pH is not less than 6.0 and not more than 11.0.

(v) Its residue on ignition is not more than 1.0 percent.

(vi) It gives positive identity tests for erythromycin stearate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH residue on ignition, identity, and crystallinity.

(ii) Samples required: A minimum of 10 containers, each consisting of 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent slurry of erythromycin stearate in water.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, 19921, May 13, 1985]

§ 452.50 Clarithromycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clarithromycin is 6-O-methylerythromycin A. It is so purified and dried that:

(i) Its potency is not less than 960 micrograms of clarithromycin activity per milligram, on an anhydrous basis.

(ii) Its moisture content is not more than 2.0 percent.

(iii) The pH of a 0.2 percent (weight per volume) slurry in aqueous methanol (95:5) is not less than 7.5 and not more than 10.0.

(iv) Its residue on ignition is not more than 0.3 percent.

(v) Its heavy metals content is not more than 20 parts per million.

(vi) Its specific rotation in chloroform containing 10 milligrams of clarithromycin per milliliter at 20 °C is between -89° and -95°, calculated on an anhydrous basis.

(vii) It gives a positive identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clarithromycin potency, moisture, pH, residue on ignition, heavy metals, specific rotation, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using a constant column temperature of 50 °C, a suitable ultraviolet detection system operating at 210 nanometers, an analytical column 3 to 30 centimeters long packed with a reversed phase packing material such as octadecyl hydrocarbon bonded silicas (3 to 10 micrometers in diameter), the inlet of this column is connected to a guard column 1 to 5 centimeters in length packed with the same material of 5- to 30-micrometer particle size, a constant flow rate of 0.7 to 1.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. The retention time for clarithromycin is between 5 and 6 minutes and the retention time for 6,11-Di-O-methylerythromycin A (resolution compound) is between 7 and 8 minutes. Mobile phase, system suitability solution, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Add 650 milliliters of methanol and 350 milliliters of 0.067 *M* potassium phosphate (monobasic) to a suitable container, mix well, and adjust the pH to 4.0 with phosphoric acid. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of system suitability solution.* Prepare a methanol solution containing approximately 625 micrograms per milliliter each of clarithromycin and 6,11-Di-O-methylerythromycin A. Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute it to volume with mobile phase to obtain a solution containing approximately 125 micrograms each of clarithromycin and 6,11-Di-O-methylerythromycin A.

(iii) *Preparation of working standard solution.* Dissolve (by shaking or sonication) an accurately weighed portion of the clarithromycin working standard in sufficient methanol to obtain a known solution containing about 625 micrograms of clarithromycin activity per milliliter. Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute to volume with mobile phase and mix to obtain a known solution containing approximately 125 micrograms of clarithromycin activity per milliliter. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter.

(iv) *Sample solution.* Dissolve (by shaking or sonication) an accurately weighed portion of the sample in sufficient methanol to obtain a solution containing 625 micrograms of clarithromycin activity per milliliter (estimated). Quantitatively transfer an aliquot of this solution to a suitable volumetric flask and dilute to volume with mobile phase and mix to obtain a known solution containing approximately 125 micrograms of clarithromycin activity per milliliter (estimated). Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter.

(v) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_S) is satisfactory if it is not less than 0.9 and not more than 1.5 for the clarithromycin peak.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 40.0 for the clarithromycin peak.

(C) *Resolution factor.* The resolution factor (R) between the peak for clarithromycin and the peak for 6,11-Di-O-methylerythromycin A is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor.* Calculate the clarithromycin capacity factor (k') as follows:

$$k' = (t_r/t_0) - 1$$

where:

t_r = Retention time of the clarithromycin peak; and

t_0 = Void volume time.

The capacity factor is satisfactory if it is not less than 1.3 and not more than 4.0. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(vi) *Calculations.* Calculate the micrograms of clarithromycin per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of clarithromycin per milligram} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m)}$$

where:

A_U = Area of the clarithromycin peak (at a retention time equal to that observed for the clarithromycin standard) in the chromatogram of the sample;

A_S = Area of the clarithromycin peak in the chromatogram of the clarithromycin working standard;

P_S = Clarithromycin activity in the clarithromycin working standard solution in micrograms per milliliter;

C_U = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(1) of that section and the titration procedure described in paragraph (e)(3) of that section, except that instead of adding 20 milliliters of solvent A before starting the titration, add a sufficient volume of solvent C to cover the electrodes in the dry titrating vessel.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, except standardize the pH meter with pH 7.0 and pH 10.0 buffers and prepare the sample as follows: Transfer 200 milligrams of the sample to a 150-milliliter beaker. Add 5 milliliters of methanol and then 95 milliliters of distilled water. Place the pH electrodes in the slurry and stir at the slowest speed possible to ensure mixing but no vortex. After 10 minutes, while still stirring, determine the pH.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(6) *Specific rotation.* Dilute an accurately weighed sample with sufficient chloroform to give a concentration of approximately 10 milligrams of clarithromycin per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube, maintaining the solution at 20 °C, and calculate the specific rotation on an anhydrous basis.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, preparing the sample as follows: Prepare a 5-percent solution of the sample in chloroform and use 0.1 millimeter matched absorption cells.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[58 FR 26653, May 4, 1993]

§ 452.60 Azithromycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Azithromycin is the dihydrate form of (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-O-methyl-α-*L*-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-*D*-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. It is so purified and dried that:

(i) Its potency is not less than 945 micrograms and not more than 1,030 micrograms of azithromycin activity per milligram, on the anhydrous basis.

(ii) Its moisture content is not less than 4.0 percent and not more than 5.0 percent.

(iii) The pH of an aqueous methanol (1:1) solution containing 2 milligrams per milliliter is not less than 9 and not more than 11.

(iv) Its residue on ignition is not more than 0.3 percent.

(v) Its heavy metals content is not more than 25 parts per million.

(vi) The specific rotation in absolute ethanol containing 20 milligrams of azithromycin per milliliter at 20 °C is between -45° to -49°, calculated on an anhydrous basis.

(vii) It is crystalline.

(viii) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for azithromycin potency, moisture, pH, residue on ignition, heavy metals, specific rotation, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an amperometric electrochemical detection system with dual glassy carbon electrodes operated in the oxidative screen mode with electrode 1 set at +0.70 volt±0.05 volt and electrode 2 at +0.82 volt±0.05 volt. (The ±0.05-volt variance allows for optimization of the background current to 70 to 100 nanoamperes.) Detection of azithromycin occurs at electrode 2 where the voltage is sufficiently high to oxidize the amine functional groups on the molecules. Use a 15-centimeters by 4.6-millimeters (inside diameter) column packed with alumina-based polybutadiene 5 micrometer spherical particles with 80-angstrom pore size (e.g., ES Industries γRP1/p). The inlet of this column is connected to a guard column 5 centimeters by 4.6 millimeters (inside diameter) packed with the same material. The flow rate is 1.5 milliliters per minute. Use a fixed volume loop injector or equivalent device to inject a volume of 50 microliters into the system. The retention time for azithromycin is between 10 and 13 minutes. Mobile phase, working standard, sample, and resolution solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Dissolve 5.8 grams of potassium phosphate monobasic in 2,130 milliliters of ultrapure deionized or high-performance liquid chromatographic-grade water. Add 870 milliliters of acetonitrile and mix. The mobile phase is 0.02 *M* potassium phosphate monobasic: acetonitrile (71:29). Adjust the pH of the mobile phase to pH 11.0±0.1 with 10 *M* potassium hydroxide (about 6 milliliters). Filter the mobile phase through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard solution.* Accurately weigh approximately 16.5 milligrams of the azithromycin working standard into a 100-milliliter volumetric flask. Dissolve the material, aided by brief sonication, in 10 milliliters of acetonitrile and dilute to volume with acetonitrile. Pipet 2.0 milliliters of this solution into a 100-milliliter volumetric flask and dilute to volume with mobile phase. This solution contains approximately 0.003 milligram per milliliter of azithromycin.

(iii) *Sample solution.* Accurately weigh approximately 16.5 milligrams of sample into a 100-milliliter volumetric flask. Dissolve the sample, aided by brief sonication, in 10 milliliters of acetonitrile and dilute to volume with acetonitrile. Pipet 2.0 milliliters of this solution into a 100-milliliter volumetric flask and dilute to volume with mobile phase.

(iv) *Resolution test solution.* Weigh approximately 16.5 milligrams each of azithromycin working standard and azaerythromycin A reference standard into a 100-milliliter volumetric flask. Dissolve the materials aided by brief sonication in 10 milliliters of acetonitrile and dilute to volume with acetonitrile. Pipet 2.0 milliliters of this solution into a 100-milliliter volumetric flask and dilute to volume with mobile phase.

(v) *System suitability requirements.* Using the resolution test solution, determine the:

(A) *Asymmetry factor.* The asymmetry factor (A_S) is satisfactory if it is not less than 0.9 and not more than 1.5 for the azithromycin peak.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 40.0 for the azithromycin peak.

(C) *Resolution factor.* The resolution factor (R) between the peak from azithromycin and the peak for azaerythromycin A is satisfactory if it is not less than 2.5.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not

more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(vi) *Calculations.* Calculate the micrograms of azithromycin per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of azithromycin per milligram} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m)}$$

where:

A_U = Area of the azithromcin peak (at a retention time equal to that observed for the azithromycin standard) in the chromatogram of the sample;

A_S = Area of the azithromcin peak in the chromatogram of the azithromycin working standard;

P_SAzithromycin activity in the azithromycin working standard solution in micrograms per milliliter;

C_U = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous methanol (1:1) solution containing 2 milligrams per milliliter, prepared by diluting a methanol solution containing 4 milligrams of azithromycin dihydrate 1:1 with distilled water.

(4) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, except use a temperature of 800 °C instead of a temperature range of 500 to 600 °C.

(5) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(6) *Specific rotation.* Dissolve an accurately weighed sample with sufficient absolute ethanol to give a concentration of approximately 20 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, except dilute and maintain the test solution at 20 °C instead of 25 °C. Use a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[58 FR 26657, May 4, 1993]

§ 452.75 Troleandomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Troleandomycin is the triacetyl ester of oleandomycin base or a mixture of two or more such esters. It is a white powder. It is so purified that:

(i) Its potency is not less than 750 micrograms of troleandomycin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in an aqueous alcohol solution containing 100 milligrams of troleandomycin per milliliter is not less than 7.0 and not more than 8.5.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) It gives a positive identity test for oleandomycin.

(vii) Its R_f value by paper chromatography is approximately 0.85. If more than one spot appears on the paper chromatogram, determine its acetyl value, which is not less than 15.3 percent and not more than 16.0 percent.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(ii) Samples of the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams, and one containing not less than 2.0 grams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical method*—(*a*) *Reagents and equipment.* (*1*) Methyl orange reagent: Shake 0.5*M* boric acid solution for 12 hours (to ensure saturation) with an excess of methyl orange indicator. An alternative method is to heat the mixture to about 50° C. and shake for about

an hour. Then allow to cool. Filter the saturated dye solution and wash three times with chloroform. Store the dye solution over chloroform.

(*2*) Acid-alcohol solution: Add 2 milliliters of concentrated sulfuric acid to 98 milliliters of absolute methyl alcohol.

(*3*) Glycerin: Reagent grade.

(*4*) Chloroform.

(*5*) Glacial acetic acid.

(*6*) Centrifuge tubes: 40 milliliters, glass-stoppered.

(*b*) *Procedure.* Using the troleandomycin working standard which has been dried for 3 hours at 60° C. and a pressure of 5 millimeters or less, prepare a standard solution in chloroform containing 50.0 milligrams of oleandomycin base in 200 milliliters. Transfer 10.0 milliliters of the solution to a 100-milliliter volumetric flask and dilute to volume with chloroform. Transfer 2.0, 4.0, 6.0, and 8.0 milliliters of this solution to glass-stoppered centrifuge tubes (40-milliliter size) and dilute to a total volume of 20.0 milliliters each with chloroform. To the 20 milliliters of the solution present in each 40-milliliter size centrifuge tube, add 0.2 milliliter of glacial acetic acid, 0.2 milliliter of glycerin, and 0.4 milliliter of methyl orange reagent. Shake for 5 minutes and centrifuge for 3 minutes. Immediately transfer to another tube a 10.0-milliliter aliquot from the chloroform (lower) layer. Care must be exercised to see that no portion of the dye-glycerin phase is included with the chloroform aliquot. Add 1.0 milliliter of acid-alcohol solution to this chloroform aliquot, mix well, and read the absorbancy at 535 nanometers, using a 1-centimeter cell and a suitable photometer and using chloroform, similarly treated, as a blank. Prepare a standard curve, plotting the absorbance values of the standard solution against the concentration expressed in micrograms of oleandomycin base per aliquot. Accurately weigh the sample to be tested to give 50 milligrams (estimated) of oleandomycin base. Dissolve in chloroform and make to 200 milliliters with chloroform. Transfer 10.0 milliliters to a 100-milliliter volumetric flask and make to volume with chloroform. Transfer 5.0 milliliters to a glass-stoppered centrifuge tube and proceed as above. Determine the potency of the sample from the standard curve.

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms per milliliter. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated solution prepared by adding 100 milligrams of troleandomycin per milliliter of water-ethyl alcohol (1:1) diluent.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter, except use a silica crucible.

(6) *Identity.* Dissolve about 10 milligrams in 5 milliliters of hydrochloric acid and heat the solution in a boiling water bath; a greenish yellow color is produced.

(7) *Rf value*—(i) *Apparatus and reagents.* (*a*) Chromatographic chamber (cylinder, glass-stoppered museum jar, 11.5 inches x 3.5 inches).

(*b*) Chromatographic paper (8 inches x 8 inches, Whatman No. 1).

(*c*) 0.1*N* hydrochloric acid.

(*d*) Resolving solvent: Butyl acetate, benzene, nitromethane, pyridine (5:5:5:1 by volume).

(*e*) Spray developing reagent: Place 1.0 milliliter of 10 percent platinic chloride solution and 25.0 milliliters of 4 percent potassium iodide solution in a 250-milliliter volumetric flask. Fill to mark with distilled water and mix well.

(ii) *Procedure.* Dissolve the sample in chloroform to give a solution containing 10 to 20 milligrams of oleandomycin base equivalent per milliliter. Prepare a sheet of chromatographic paper by drawing a line of origin parallel to and 1 inch from the edge of the paper. Wet the paper thoroughly with the 0.1*N* hydrochloric acid and blot it firmly between

sheets of absorbent paper. Starting 2 inches in from the edge and at 1-inch intervals, apply 3 to 5 microliters of the sample solutions to the starting line. Allow a few minutes for the paper to dry partially. While it is still damp, form a cylinder by bringing the outer edges together, allowing about 1-inch overlap, and secure with a paper clip. Stand the paper in the chromatographic chamber, which has been filled to a depth of one-half of an inch with the resolving solvent. After the solvent front rises to a height of 4 to 5 inches above the origin, remove the paper from the tank and hang it up to air dry. Spray the dried paper with the developing reagent. Hang the paper in a 100° C. oven for 3 minutes. A purple spot becomes visible for troleandomycin at an R_f value of about 0.85. The approximate R_f values for diacetyloleandomycin, monoacetyloleandomycin, and oleandomycin are, respectively, 0.72, 0.27, and 0.13.

(8) *Acetyl determination*—(i) *Apparatus and reagents.* (*a*) One 3-necked Pyrex flask of approximately 45 milliliters capacity, pear-shaped with T-joints, agar inlet tube, glass-stoppered funnel, glass condenser, and bubble counter.

(*b*) 50-milliliter Pyrex Erlenmeyer flask.

(*c*) 10-milliliter buret, calibrated to 0.02 milliliter.

(*d*) Anhydrous methyl alcohol, reagent grade.

(*e*) 2*N* sodium hydroxide solution.

(*f*) Sulfuric acid solution prepared by adding 100 milliliters of concentrated H_2SO_4 to 200 milliliters of water.

(*g*) 1*N* barium chloride solution.

(*h*) Phenolphthalein solution (1 percent in ethyl alcohol).

(*i*) Water-pumped nitrogen.

(*j*) NaOH solution 0.015*N*.

(ii) *Procedure.* Weigh accurately (to 0.01 milligram) approximately 30 milligrams of the sample into the three-necked acetyl flask. Add 2.0 milliliters of methyl alcohol to dissolve the sample; then add slowly, with gentle swirling, 1.0 milliliter of NaOH solution. Connect the gas inlet tube with bubble counter attached and adjust nitrogen flow to about two bubbles a second. Put glass-stoppered funnel in centerneck of acetyl flask and put about 5 milliliters of H_2O in the funnel. Add a boiling chip to the solution and attach condenser in the refluxing position with water cooling. Adjust burner flame under acetyl flask to reflux solution gently. Reflux for 30 minutes. Cool assembly slightly; then rinse down condenser (still in reflux position) with a few milliliters of H_2O. Reassemble condenser to the distillation position and add water through the funnel to make a total of approximately 5 milliliters of H_2O added to acetyl flask. Adjust burner flame so that about 5 milliliters of H_2O and methyl alcohol is distilled over in approximately 10 minutes. Discard this distillate. Cool acetyl flask slightly. Acidify solution in flask by adding 1 milliliter of the sulfuric acid solution through the funnel. Adjust burner flame and distill over approximately 20 milliliters of distillate into an Erlenmeyer flask in about 20 minutes, adding water through the funnel as necessary. It is important to keep the liquid volume in the acetyl flask around 2 to 3 milliliters in order to obtain a quantitative recovery of the acetic acid. Collect a second fraction of distillate, about 10 milliliters in volume. As the second fraction is distilling, process the first fraction. Heat the first fraction and boil gently about 20 seconds. Add a few drops of $BaCl_2$ solution to check if any sulfate was distilled over. If the sulfate is present, discard and repeat the whole determination. If the sulfate is absent, immediately titrate the solution with the 0.015*N* NaOH solution to a faint-pink endpoint, using one drop of phenolphthalein solution as the indicator. Repeat the above procedure with the second fraction. If the second fraction requires less than 0.10 milliliter of the 0.015*N* NaOH solution and all the acetic acid has been distilled over, the determination is completed. If greater than this, collect a third fraction of approximately 10 milliliters and titrate this as before. Total volumes of NaOH used and calculate results as follows:

(Milliliters of NaOH×*N* NaOH×0.043×100)/(Weight sample in grams)=Percent acetyl.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19920, 19921, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 452.110 Erythromycin oral dosage forms.

§ 452.110a Erythromycin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin tablets are erythromycin with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, flavorings, and suitable preservatives. The potency of each tablet is 250 milligrams or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Tablets shall disintegrate within 1 hour. The loss on drying is not more than 5.0 percent. The erythromycin used in making the batch conforms to the standards prescribed by § 452.10(a)(1), except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, disintegration time, and loss on drying.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

[39 FR 19149, May 30, 1974, as amended at 42 FR 59068, Nov. 15, 1977; 50 FR 19921, May 13, 1985]

§ 452.110b Erythromycin enteric-coated tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromyin enteric-coated tablets are enteric-coated tablets composed of erythromycin, suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 100, 250, 333, or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The tablets shall disintegrate within 2 hours. The moisture content is not more than 6 percent. The erythromycin base used in making the batch conforms to the standards of § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the procedure described in paragraph (e)(3) of that section.

[39 FR 14149, May 30, 1974, as amended at 44 FR 30332, May 25, 1979; 44 FR 48190, Aug. 17, 1979; 46 FR 44442, Sept. 4, 1981; 47 FR 15326, Apr. 9, 1982; 50 FR 19921, May 13, 1985]

§452.110c Erythromycin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin capsules are capsules containing enteric-coated erythromycin pellets, suitable and harmless buffer substances, diluents, binders, lubricants, and colorings. Each capsule contains either 125 milligrams or 250 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 7.5 percent. It passes the acid resistance/dissolution test. The erythromycin used conforms to the standards prescribed by §452.10(a)(1), except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, moisture, and acid resistance/dissolution.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol. Blend for 2 to 3 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute an aliquot with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter, using the sample preparation method described in paragraph (d)(1) of that section.

(3) *Acid resistance/dissolution.* Proceed as directed in §436.542 of this chapter. The quantity Q (the amount of erythromycin dissolved) is 85 percent at 45 minutes.

[46 FR 16678, Mar. 13, 1981; 46 FR 22359, Apr. 17, 1981, as amended at 50 FR 19921, May 13, 1985; 50 FR 36992, Sept. 11, 1985; 50 FR 47214, Nov. 15, 1985]

§452.110d Erythromycin particles in tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin particles in tablets are tablets containing erythromycin acid-resistant coated particles, suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains 333 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The loss on drying is not more than 5.0 percent. It passes the dissolution test and the acid resistance test. The erythromycin used conforms to the standards prescribed by §452.10(a)(1), except heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, safety, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, dissolution, and acid resistance.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The erythromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing 200 milliliters of methyl alcohol. Blend for 2 to 3 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute an aliquot with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity *Q* (the amount of erythromycin dissolved) is 75 percent at 45 minutes.

(4) *Acid resistance.* Proceed as directed in § 436.545 of this chapter. The quantity of erythromycin dissolved is not more than 25 percent at 60 minutes.

[51 FR 37723, Oct. 24, 1986, as amended at 55 FR 11584, Mar. 29, 1990]

§ 452.115 Erythromycin estolate oral dosage forms.

§ 452.115a Erythromycin estolate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate tablets are composed of erythromycin estolate with one or more suitable and harmless diluents, binders, lubricants, and colorings. Each tablet contains erythromycin estolate equivalent to 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The tablets shall disintegrate within 30 minutes. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch. A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tables into a high-speed glass blender jar with 200 milliliters of methyl alcohol. Blend for 3 to 5 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Hydrolyze a portion of this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§452.115b Erythromycin estolate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, quality, and purity.* Erythromycin estolate capsules are capsules containing erythromycin estolate with suitable and harmless buffer substances and diluents enclosed in a gelatin capsule. The erythromycin estolate content of each capsule is equivalent to either 250 milligrams of erythromycin or 125 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The erythromycin estolate used conforms to the standards prescribed therefor by §452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain;

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Test and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender with 200 milliliters of methyl alcohol for 2 to 3 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Hydrolyze a portion of this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§452.115c Erythromycin estolate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate oral suspension is erythromycin estolate with suitable and harmless buffer substances, dispersing agents, diluents, coloring, and flavorings. Each milliliter contains erythromycin estolate equivalent to 25, 50, or 100 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 3.5 and not more than 6.5. The erythromycin estolate used conforms to the standards prescribed by §452.15(a)(1).

(2) *Labeling.* In addition to conforming with the requirements of §432.5 of this chapter, each package shall bear on its outside wrapper or container and the immediate container the statement ''Refrigerate'' or ''Keep under refrigeration''.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 containers, each having not less than 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Remove an accurately measured representative volume of the suspension and dilute with sufficient methyl alcohol to give a concentration of 2.5 milligrams per milliliter (estimated). Dilute the entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a

concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug as it is prepared for dispensing.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 452.115d Erythromycin estolate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for oral suspension is a dry mixture of erythromycin estolate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. The erythromycin estolate content is 25 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. When reconstituted as directed in its labeling, its pH is not less than 5.0 and not more than 7.0. Its moisture content is not more than 2.0 percent. The erythromycin estolate used conforms to the standards of § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, moisture, pH, crystallinity, and identity.

(*b*) The batch: Potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 immediate containers, each consisting of 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in its labeling.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 452.115e Erythromycin estolate for pediatric drops.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for pediatric drops is a dry mixture of erythromycin estolate with suitable and harmless dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains the equivalent of 100 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. Its moisture content is not more than 2.0 percent. Its pH is not less than 5.0 nor more than 5.5. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of

§431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Withdraw an accurately measured representative volume of the reconstituted suspension and add sufficient methyl alcohol to give a concentration of 2.5 milligrams of erythromycin base per milliliter (estimated). Dilute this entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

(3) *pH*. Proceed as directed in §436.202(b) of this chapter, using the suspension prepared as directed in the labeling.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§452.115f Erythromycin estolate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin estolate chewable tablets are tablets composed of erythromycin estolate and suitable and harmless diluents, binders, buffers, colorings, and flavorings. Each tablet contains erythromycin estolate equivalent to either 125 or 250 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 4 percent. The erythromycin estolate used in making the batch conforms to the standards prescribed by §452.15(a)(1).

(2) *Labeling*. It shall be labeled in accordance with §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each consisting of not less than 300 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes in 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Hydrolyze this solution in a 60° C. constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§452.115g Erythromycin estolate and sulfisoxazole acetyl oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin estolate and sulfisoxazole acetyl oral suspension is erythromycin estolate and sulfisoxazole acetyl with suitable and harmless buffer substances, preservatives, solvents, stabilizers, emulsifiers, dispersing agents, colorings, and

flavorings. Each milliliter contains erythromycin estolate equivalent to 25 milligrams of erythromycin and 120 milligrams of sulfisoxazole. Its erythromycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its sulfisoxazole acetyl content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of sulfisoxazole that it is represented to contain. Its pH is not less than 3.5 and not more than 6.5. The erythromycin estolate used conforms to the standards prescribed by § 452.15(a)(1). The sulfisoxazole acetyl used conforms to the standards prescribed by the U.S.P. XXII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The erythromycin estolate used in making the batch for potency, moisture, pH, crystallinity, and identity.

(B) The sulfisoxazole acetyl used in making the batch for all U.S.P. XXII specifications.

(C) The batch for erythromycin content, sulfisoxazole content, and pH.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(B) The batch: a minimum of 15 immediate containers.

(b) *Tests and methods of assay*—(1) *Erythromycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove an accurately measured representative volume of the suspension and dilute with sufficient methyl alcohol to give a concentration of 2.5 milligrams per milliliter (estimated). Dilute the entire mixture with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Hydrolyze in a 60 °C constant temperature water bath for 2 hours or at room temperature for 16 to 18 hours. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sulfisoxazole content.* Proceed as directed in § 436.328 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug as it is prepared for dispensing.

[55 FR 280, Jan. 4, 1990]

§ 452.125 Erythromycin ethylsuccinate oral dosage forms.

§ 452.125a Erythromycin ethylsuccinate chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate chewable tablets are composed of erythromycin ethylsuccinate and suitable and harmless diluents, binders, buffers, colorings, and flavorings. Each tablet contains erythromycin ethylsuccinate equivalent to 200 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 5 percent. The erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''erythromycin ethylsuccinate tablets''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender for 2 to 3 minutes with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 41 FR 51596, Nov. 23, 1976; 42 FR 29858, June 10, 1977; 50 FR 19921, May 13, 1985]

§ 452.125b Erythromycin ethylsuccinate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin ethylsuccinate oral suspension is erythromycin ethylsuccinate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, flavorings, and preservatives. Each milliliter contains erythromycin ethylsuccinate equivalent to 40 or 80 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 6.5 and not more than 8.5. The erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, identity, residue on ignition, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers each consisting of 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative volume of the suspension into a high-speed glass blender jar and add sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 452.125c Erythromycin ethylsuccinate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin ethylsuccinate for oral suspension is a dry mixture of erythromycin ethylsuccinate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 40 milligrams or 80 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its loss on drying is not more than 1 percent. When reconstituted as directed in the labeling, its pH is not less than 7.0 nor more than 9.0. The crystalline erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency,

moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch, for potency, pH, and loss on drying.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers each consisting of approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Place an accurately measured representative portion of the sample into a high-speed glass blender jar containing sufficient methyl alcohol to give a final volume of 200 milliliters. Blend for 3 to 5 minutes. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension prepared as directed in the labeling. If the suspension contains 80 milligrams per milliliter, equilibrium usually is reached in approximately 15 minutes.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 47 FR 21240, May 18, 1982; 50 FR 19921, May 13, 1985]

§ 452.125d Erythromycin ethylsuccinate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate tablets are composed of erythromycin ethylsuccinate and suitable and harmless diluents, binders, buffers, and colorings. Each tablet contains erythromycin ethylsuccinate equivalent to 400 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The loss on drying is not more than 4.0 percent. The tablets shall disintegrate within 40 minutes. The erythromycin ethylsuccinate used conforms to the standards prescribed by § 452.25(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient methyl alcohol to yield a concentration of 5 milligrams of erythromycin activity or less per milliliter when blended. Blend for 3 to 5 minutes. Further dilute an aliquot of this solution with 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *Disintegration time*—(i) *If the tablet is uncoated.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

(ii) *If the tablet is plain-coated.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

[41 FR 51596, Nov. 23, 1976, as amended at 50 FR 19921, May 13, 1985; 55 FR 14091, Apr. 16, 1990]

§ 452.125e Erythromycin ethylsuccinate-sulfisoxazole acetyl for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate-sulfisoxazole acetyl for

oral suspension is a dry mixture of erythromycin ethylsuccinate and sulfisoxazole acetyl with suitable and harmless flavorings, buffers, surfactants, colorings, and suspending agents. When reconstituted as directed in the labeling, each milliliter will contain erythromycin ethylsuccinate equivalent to 40 milligrams of erythromycin and sulfisoxazole acetyl equivalent to 120 milligrams of sulfisoxazole. Its erythromycin ethylsuccinate content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its sulfisoxazole acetyl content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of sulfisoxazole that it is represented to contain. Its loss on drying is not more than 1.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.0. The erythromycin ethylsuccinate used conforms to the standards prescribed by §452.25(a)(1). The sulfisoxazole acetyl used conforms to the standards prescribed by the U.S.P.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The sulfisoxazole acetyl used in making the batch for all U.S.P. specifications.

(*c*) The batch for erythromycin content, sulfisoxazole content, loss on drying, and pH.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Erythromycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Allow to stand for 1 hour. Shake gently and transfer 5 milliliters of the well-shaken suspension into a high-speed glass blender jar containing 195 milliliters of methyl alcohol. Blend for 3 to 5 minutes. Further dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sulfisoxazole acetyl content.* Proceed as directed in §436.328 of this chapter.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the suspension reconstituted as directed in the labeling.

[46 FR 2990, Jan. 13, 1981, as amended at 50 FR 19921, May 13, 1985]

§452.135 Erythromycin stearate oral dosage forms.

§452.135a Erythromycin stearate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate tablets are tablets composed of erythromycin stearate with suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains erythromycin stearate equivalent to 75, 100, 125, 250, or 500 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Tablets shall disintegrate within 1½ hours. The loss on drying is not more than 5.0 percent. The erythromycin stearate used in making the tablets conforms to the standards prescribed by §452.35(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, pH,

moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender with 200 milliliters of methyl alcohol for 3 to 5 minutes. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(2) of that section.

[39 FR 19149, May 30, 1974, as amended at 42 FR 59068, Nov. 15, 1977; 50 FR 19921, May 13, 1985]

§ 452.135b Erythromycin stearate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate oral suspension is erythromycin stearate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 25 milligrams of erythromycin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its pH is not less than 7.0 and not more than 8.5. The erythromycin stearate used conforms to the standards prescribed by § 452.35(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative volume of the suspension into a high-speed glass blender jar. Add sufficient methyl alcohol to the jar to give a concentration of 1.25 milligrams of erythromycin base per milliliter (estimated). Blend for 2 to 3 minutes. Add sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a concentration of 0.5 milligrams of erythromycin base per milliliter (estimated) and blend again for 2 to 3 minutes. Further dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 452.135c Erythromycin stearate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate for oral suspension is a dry mixture of erythromycin stearate with suitable and harmless buffer substances, dispersing agents, diluents, colorings, and flavorings. It contains the equivalent of 25 or 50 milligrams of erythromycin per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. Its moisture content is not more than 2 percent. When reconstituted as directed in the

labeling, its pH is not less than 6.0 and not more than 9.0. The erythromycin stearate used conforms to the standards prescribed by §452.35(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin stearate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin stearate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Place an accurately measured representative portion of the suspension into a high-speed glass blender jar with sufficient methyl alcohol to give a concentration of 1.0 milligram of erythromycin base per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the suspension obtained when the drug is reconstituted as directed in the labeling.

[40 FR 49083, Oct. 21, 1975, as amended at 41 FR 24884, June 21, 1976; 50 FR 19921, May 13, 1985]

§452.150 Clarithromycin oral dosage forms.

§452.150a Clarithromycin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clarithromycin tablets are composed of clarithromycin and one or more suitable and harmless diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 250 milligrams or 500 milligrams of clarithromycin activity. Its clarithromycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clarithromycin that it is represented to contain. The loss on drying is not more than 6.0 percent. It passes the dissolution test. It passes the identity test. The clarithromycin used conforms to the standards prescribed by §452.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clarithromycin used in making the batch for potency, moisture, pH, residue on ignition, heavy metals, specific rotation, identity, and crystallinity.

(B) The batch for content, loss on drying, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clarithromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Clarithromycin content.* Proceed as directed in §452.50(b)(1), preparing the sample solution and calculating the clarithromycin content as follows:

(i) *Preparation of sample solution.* Grind and composite five whole tablets in a glass mortar and pestle and quantitatively transfer the powder to a 500-milliliter volumetric flask with 50 milliliters of distilled water and shake mechanically until the tablets are well dispersed (approximately 5 to 10 minutes). Add 300 milliliters of methanol and shake mechanically for 30 minutes. Dilute with methanol to volume and mix. Allow the excipients to settle. Quantitatively transfer and dilute a convenient aliquot of the supernatant with mobile phase (described in §452.50(b)(1)(i)) to obtain a solution

containing 125 micrograms of clarithromycin per milliliter (estimated). Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter.

(ii) *Calculations.* Calculate the clarithromycin content as follows:

$$\text{Milligrams of clarithromycin per tablet} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_U = Area of the clarithromycin peak (at a retention time equal to that observed for the clarithromycin standard) in the chromatogram of the sample;

A_S = Area of the clarithromycin peak in the chromatogram of the clarithromycin working standard;

P_S = Clarithromycin activity in the clarithromycin working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of tablets in the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(c) of this chapter, using a sample weight of 1 to 2 grams.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the amount of clarithromycin dissolved) is 80 percent at 30 minutes.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention time for the peak of the active ingredient must be within 2 percent of the retention time for the peak of the corresponding reference standard.

[58 FR 26654, May 4, 1993. Redesignated at 61 FR 34726, July 3, 1996]

§ 452.150b Clarithromycin granules for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clarithromycin granules for oral suspension is a dry mixture containing clarithromycin-coated particles, suitable and harmless dispersing agents, diluents, preservatives, and flavorings. It contains the equivalent of 25 or 50 milligrams of clarithromycin activity per milliliter of the reconstituted suspension. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of clarithromycin that it is represented to contain. Its loss on drying is not more than 2.0 percent. When constituted as directed in the labeling, its pH is not less than 4.0 nor more than 5.4. The clarithromycin used conforms to the standards prescribed by § 452.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clarithromycin used in making the batch for potency, moisture, pH, residue on ignition, heavy metals, specific rotation, identity, and crystallinity.

(B) The batch for content, loss on drying, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clarithromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clarithromycin content.* Proceed as directed in § 452.50(b)(1), except use a known injection volume between 10 and 60 microliters. Also, prepare the mobile phase, working standard solution, and sample solution, and use system suitability requirements and calculation as follows:

(i) *Mobile phase.* Add 600 milliliters of methanol and 400 milliliters of 0.067M potassium phosphate, monobasic, to a suitable container, mix well, and adjust the pH to 3.5 with phosphoric acid. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just before its introduction into the chromatographic system.

(ii) *Preparation of standard solution.* Dissolve an accurately weighed portion of the clarithromycin working standard in sufficient methanol to obtain a solution having a known concentration of approximately 2.1 milligrams per milliliter of clarithromycin. Quantitatively transfer and dilute an aliquot of this solution with mobile phase and mix to obtain a solution of known

concentration of approximately 415 micrograms of clarithromycin per milliliter.

(iii) *Preparation of sample solution.* Constitute as directed in the labeling. Accurately measure a representative portion of the suspension that contains about 1 to 2 grams of clarithromycin activity and, using approximately 330 milliliters of 0.067M potassium phosphate, dibasic, quantitatively transfer into a 1,000 milliliter volumetric flask containing approximately 50 milliliters of 0.067M potassium phosphate, dibasic. Shake for 30 minutes. Dilute to volume with methanol. Mix well and place in an ultrasonic bath for 30 minutes. Cool to room temperature and adjust to volume with methanol. Add a magnetic stirring bar and stir for 60 minutes. Allow excipients to settle and dilute an appropriate aliquot of the solution with mobile phase to obtain a solution containing 500 micrograms of clarithromycin activity per milliliter and mix well. Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter.

(iv) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (T) is satisfactory if it is not less than 1.0 and not greater than 1.7 for the clarithromycin peak.

(B) *Efficiency of the column.* The efficiency (n) is satisfactory if it is greater than 2,100 theoretical plates for the clarithromycin peak.

(C) *Capacity factor.* The capacity factor (k') is satisfactory if it is between 2.5 and 6 for the clarithromycin peak.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of three replicate injections) is satisfactory if it is not more than 2.0 percent.

(v) *Calculations.* Calculate the clarithromycin content as follows:

$$\text{Milligrams of clarithromycin per milliliter} = \frac{A_U \times P_S \times D}{A_S \times V}$$

where:

A_U = Area of the clarithromycin peak in the chromatogram of the sample;

A_S = Area of the clarithromycin peak in the chromatogram of the clarithromycin working standard;

P_S = Clarithromycin activity in the clarithromycin working standard solution in micrograms per milliliter;

D = Dilution factor of the sample test solution; and

V = Volume, in milliliters, of the portion of suspension taken.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, using a sample weight of approximately 1 gram, weighing in a normal laboratory atmosphere.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the suspension prepared as directed in the labeling. Stir the suspension for 10 minutes with the electrode immersed and record the pH.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention times for the clarithromycin peak must be within 2 percent of the retention time for the peak of the reference standard.

[61 FR 34726, July 3, 1996]

§ 452.160 Azithromycin oral dosage forms.

§ 452.160a Azithromycin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Azithromycin capsules are composed of azithromycin and one or more suitable and harmless diluents, disintegrants, lubricants, and wetting agents enclosed in a gelatin capsule. Each capsule contains azithromycin equivalent to 250 milligrams of azithromycin. The azithromycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of azithromycin that it is represented to contain. The moisture content of the capsules is not more than 5.0 percent. The capsules pass the dissolution test.

The capsules pass the identity test. The azithromycin used conforms to the standards prescribed by § 452.60(a)(1) of this part.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain;

(i) Results of tests and assays on:

(A) The azithromycin used in making the batch for potency, moisture, pH, residue on ignition, heavy metals, specific rotation, crystallinity, and identity.

(B) The batch for content, moisture, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The azithromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Azithromycin content.* Proceed as directed in § 452.60(b)(1), preparing the sample solution and calculating the azithromycin content as follows:

(i) *Preparation of sample solution.* Quantitatively transfer the contents of one capsule into a 250-milliliter volumetric flask. Add about 175 milliliters of acetonitrile and shake on a reciprocating shaker for 30 minutes. Dilute to volume with acetonitrile, stopper the flask and mix well. Place 40 milliliters of the resulting suspension into a suitably sized centrifuge tube. Stopper the tube and centrifuge the suspension (about 10 minutes at 1,000 rpm). Pipet 2.0 milliliters of the supernatant into a 50-milliliter volumetric flask and dilute to volume with the mobile phase. Pipet 2.0 milliliters of this solution into a 25-milliliter volumetric flask and dilute to volume with mobile phase. The final dilution of the sample and standard must be identical. The final concentration of azithromycin in the sample solution is 0.003 milligram per milliliter (estimated).

(ii) *Calculations.* Calculate the azithromycin content as follows:

$$\text{Milligrams of azithromycin per capsule} = \frac{A_U \times P_S \times d}{A_S \times 1{,}000}$$

where:

A_U = Area of the azithromycin peak (at a retention time equal to that observed for the azithromycin standard) in the chromatogram of the sample;

A_S = Area of the azithromycin peak of the azithromycin working standard;

P_S = Azithromycin activity in the azithromycin working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample = 250×50×25/2×2.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity Q (the percentage of azithromycin activity dissolved) is 75 percent within 45 minutes.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section the retention time for the peak of the active ingredient must be with 2 percent of the retention time for the peak of the corresponding reference standard.

[58 FR 26658, May 4, 1993. Redesignated at 59 FR 52078, Oct. 14, 1994.]

§ 452.160b Azithromycin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Azithromycin for oral suspension is a dry mixture of azithromycin with a suitable and harmless buffer substance, sweetener, diluent, anticaking agent, and flavorings. The dry mixture is packaged in single dose packets each containing 1,000 milligrams of azithromycin. The azithromycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of azithromycin that it is represented to contain. Its moisture content is not more than 1.5 percent. When constituted as directed in the labeling, the pH of the suspension is not less than 9 and not more than 11. It gives a positive identity test for azithromycin. The azithromycin used conforms to the standards prescribed by § 452.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain;

(i) Results of tests and assays on:

(A) The azithromycin used in making the batch for potency, moisture, pH, residue on ignition, heavy metals, specific rotation, crystallinity, and identity.

(B) The batch for content, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The azithromycin used in making the batch: 10 packages, each containing approximately 1,000 milligrams.

(B) The batch: A minimum of 30 packages.

(b) *Tests and methods of assay*—(1) *Azithromycin content.* Proceed as directed in § 452.60(b)(1), preparing the dissolving solvent and sample solution and calculating the azithromycin content as follows:

(i) *Dissolving solvent.* Dissolve 2.2 grams of potassium phosphate monobasic in 1,590 milliliters of ultrapure deionized or high-performance liquid chromatographic-grade water. Add 600 milliliters of 2-propanol, 480 milliliters of ethanol, and 330 milliliters of acetonitrile, adjust to pH 8.4 with 10*M* potassium hydroxide and shake on a reciprocating shaker for 30 minutes. The dissolving solvent is 0.01*M* monobasic potassium phosphate:2-propanol:ethanol:acetonitrile (53:20:16:11, by volume).

(ii) *Preparation of sample solution.* Quantitatively transfer the contents of one package into a 500-milliliter volumetric flask. Add about 350 milliliters of dissolving solvent and shake on a reciprocating shaker for 30 minutes. Dilute to volume with dissolving solvent, stopper the flask, and mix well. Place 40 milliliters of the resulting suspension into a suitably sized centrifuge tube. Stopper the tube and centrifuge the suspension (about 20 minutes at 1,000 revolutions per minute). Pipet 10.0 milliliters of the diluted solution into a 50-milliliter volumetric flask and dilute to volume with mobile phase (described in § 452.60(b)(1)(i)). Pipet 2.0 milliliters of the diluted solution into a 50-milliliter volumetric flask and dilute to volume with mobile phase. The final dilution of the sample and standard must be identical. The final concentration of azithromycin in the sample solution is 0.003 milligram per milliliter (estimated).

(iii) *Calculations.* Calculate the azithromycin content as follows:

$$\text{Milligrams of azithromycin per package} = \frac{A_U \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_U = Area of the azithromycin peak in the chromatogram of the sample (at a retention time equal to that observed for the azithromycin standard);

A_S = Area of the azithromycin peak in the chromatogram of the azithromycin working standard;

P_S = Azithromycin activity in the azithromycin working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample = 500 X 50/10 X 50/10 X 50/2.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug constituted as directed in the labeling. Allow the constituted suspension to sit for 10 minutes undisturbed before making the measurement.

(4) *Identity.* Using the high-performance liquid chromatographic procedure described in paragraph (b)(1) of this section, the retention time for the peak of the active ingredient must be within 2 percent of the retention time for the peak of the corresponding reference standard.

[59 FR 52078, Oct. 14, 1994]

§ 452.175 Troleandomycin oral dosage forms.

§ 452.175a Troleandomycin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Troleandomycin capsules are capsules composed of troleandomycin and one or more suitable buffers, diluents, binders, lubricants, and colorings. Each capsule contains 125 milligrams or 250 milligrams

of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of troleandomycin that it is represented to contain. The loss on drying is not more than 5 percent. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§ 452.175b Troleandomycin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Troleandomycin oral suspension is troleandomycin and one or more suitable buffers, dispersants, flavorings, colorings, and preservatives suspended in a suitable and harmless vehicle. Each milliliter contains 25 milligrams of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of troleandomycin that it is represented to contain. Its pH is not less than 5.0 and not more than 8.0. The troleandomycin used conforms to the standards prescribed by § 452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§452.175c Troleandomycin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Troleandomycin for oral suspension is troleandomycin with suitable buffers, dispersants, preservatives, colorings, and flavorings. When the suspension is prepared as directed in its labeling, each milliliter contains 25 milligrams of troleandomycin. However, if it is for pediatric use, each milliliter contains 100 milligrams of troleandomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of troleandomycin that it is represented to contain. Its loss on drying is not more than 2 percent. The pH of the suspension, when prepared as directed in its labeling, is not less than 5.0 and not more than 7.0. The troleandomycin used conforms to the standards prescribed by §452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately equal portions of not less than 500 milligrams and one containing not less than 2 grams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the drug as directed in the labeling. Dilute an accurately measured representative portion of the sample with sufficient 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using the suspension obtained after reconstituting the drug as directed in its labeling.

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§452.175d Troleandomycin chewable tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Each troleandomycin chewable tablet contains an amount equivalent to 125 milligrams of troleandomycin with suitable diluents, binders, buffers, colorings, and flavorings. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of troleandomycin that it is represented to contain. The loss on drying is not more than 5 percent. The troleandomycin used conforms to the standards prescribed by §452.75(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The troleandomycin used in making the batch for potency, loss on drying, pH, residue on ignition, identity, R_f value, acetyl value (only if more than one spot is present in the determination of R_f value), and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The troleandomycin used in making the batch: 10 packages, nine containing approximately 500 milligrams each and one containing approximately 2 grams.

(*b*) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar with sufficient 80 percent isopropyl alcohol solution (solution 15) to obtain a stock solution containing 1,000 micrograms of troleandomycin per milliliter (estimated). Blend 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 25 micrograms of troleandomycin per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 48 FR 36571, Aug. 12, 1983; 50 FR 19921, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 452.225 Erythromycin ethylsuccinate injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin ethylsuccinate injection is erythromycin ethylsuccinate and butylaminobenzoate dissolved in polyethylene glycol 400. It contains a suitable and harmless preservative. Each milliliter contains 50 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin that it is represented to contain. It contains 2 percent butylaminobenzoate. It is sterile. Its moisture content is not more than 1.5 percent. The erythromycin ethylsuccinate used conforms to the standards prescribed therefore by § 452.25a(a)(1).

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5 of this chapter, each immediate container shall bear on its label and labeling the statement: ''Warning—For intramuscular use only''.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation, except that if the product is sterilized after filling, a representative sample consisting of 10 immediate containers from each sterilizer load. If only one sterilizer load is involved, the sample shall consist of 20 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: By means of a suitable hypodermic needle and syringe, remove an accurately measured representative volume of the sample and dilute with sufficient methyl alcohol to give a solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use a bacterial-retentive membrane resistant to the solvent polyethylene glycol 400 and add 1 milliliter from each immediate container directly to the membrane, thus eliminating the preliminary solubilization step.

(3) [Reserved]

(4) *Moisture.* Proceed as directed in § 436.201(e)(1) of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, 19921, May 13, 1985]

§ 452.230 Sterile erythromycin gluceptate.

The requirements for certification and the tests and methods of assay for sterile erythromycin gluceptate packaged for dispensing are described in § 452.30a.

[46 FR 16685, Mar. 13, 1981]

§ 452.232 Erythromycin lactobionate injectable dosage forms.

§ 452.232a Erythromycin lactobionate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate for injection is a dry mixture of erythromycin lactobionate and a suitable preservative. It contains the equivalent of 300 milligrams, 500 milligrams, or 1 gram of erythromycin per vial. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 5 percent. Its pH is not less than 6.5 and not more than 7.5. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1) (i), (iii), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, heavy metals, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the total withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the preparation, withdraw an accurately measured volume from each container. Dilute with sterile distilled water to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 30 milligrams of erythromycin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 50 milligrams of erythromycin per milliliter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19920, 19921, May 13, 1985. Redesignated at 51 FR 35216, Oct. 2, 1986]

§ 452.232b Sterile erythromycin lactobionate.

The requirements for certification and the tests and methods of assay for sterile erythromycin lactobionate packaged for dispensing are described in § 452.32a.

[51 FR 35216, Oct. 2, 1986]

Subpart D—Ophthalmic Dosage Forms

§ 452.310 Erythromycin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ophthalmic ointment is erythromycin in a suitable and harmless ointment base. Each gram of ointment contains 5 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. It is sterile. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: Twenty immediate containers, collected at regular intervals throughout each filling operation.

(b)(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment in a separatory funnel containing 50 milliliters of reagent-grade petroleum ether. Shake until dissolved. Wash with four separate washings of a 4:1 mixture of methyl alcohol and distilled water. Combine the washings and bring to volume with the methyl alcohol-water solution in a volumetric flask. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 49 FR 5097, Feb. 10, 1984; 50 FR 19921, May 13, 1985]

Subpart E [Reserved]

Subpart F—Dermatologic Dosage Forms

§ 452.510 Erythromycin dermatologic dosage forms.

§ 452.510a Erythromycin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ointment is erythromycin in a suitable and harmless ointment base. It may contain suitable preservatives. Each gram of ointment contains 20 milligrams of erythromycin. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1) (i), (iii), (iv), (v), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, residue on ignition, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the ointment in a separatory funnel containing 50 milliliters of reagent-grade petroleum ether. Shake until dissolved. Wash with four separate washings of a 4:1 mixture of methyl alcohol and distilled water. Combine the washings and bring to volume with the methyl alcohol-water solution in a volumetric flask. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture*. Proceed as directed in §436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 49 FR 5097, Feb. 10, 1984; 49 FR 47829, Dec. 7, 1984; 50 FR 47214, Nov. 15, 1985]

§452.510b Erythromycin topical solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Erythromycin topical solution contains in each milliliter 15.0 or 20.0 milligrams of erythromycin. It may also contain one or more suitable and harmless solvents, surfactants, buffer substances, diluents, and perfumes. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. If it contains 15.0 milligrams of erythromycin per milliliter, its moisture content is not more than 5.0 percent. If it contains 20.0 milligrams of erythromycin per milliliter, its moisture content is not more than 8.0 percent, except if it contains acetone, its moisture content is not more than 2.0 percent. The erythromycin used conforms to the standards prescribed by §452.10(a)(1), except heavy metals.

(2) *Packaging*. In addition to the requirements of §432.1 of this chapter, it may either be dispensed on individually packaged pledgets, each individual pledget containing 0.8 milliliter of erythromycin topical solution, or in a jar containing 60 pledgets. The jar contains 0.8 milliliter of erythromycin topical solution per pledget. Although the pledgets in the jar are not individually packaged, the drug is uniformly distributed throughout the pledgets. The erythromycin topical solution used on the pledgets contains 20 milligrams of erythromycin per milliliter.

(3) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(4) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*. If the erythromycin topical solution is dispensed on a pledget, express the contents of a representative number of pledgets into a suitable container to obtain a volume of sample adequate to perform each assay described in paragraph (b)(1) and (2) of this section.

(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion of the sample and dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture*. Proceed as directed in §436.201 of this chapter, except if the

sample contains acetone, in lieu of Solvent A, use a mixture of pyridine and methanol (1:1).

[46 FR 2995, Jan. 13, 1981, as amended at 48 FR 51293, Nov. 8, 1983; 49 FR 374, Jan. 4, 1984; 50 FR 1504, Jan. 11, 1985; 50 FR 19921, May 13, 1985; 50 FR 20204, May 15, 1985; 54 FR 47352, Nov. 14, 1989; 54 FR 50472, Dec. 6, 1989]

§ 452.510d Erythromycin-benzoyl peroxide topical gel.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-benzoyl peroxide topical gel is erythromycin packaged in combination with a suitable and harmless gel containing benzoyl peroxide and one or more suitable dispersants, stabilizing agents, perfumes, and wetting agents. When reconstituted as directed in the labeling, each gram contains 30 milligrams of erythromycin and 50 milligrams of benzoyl peroxide. The erythromycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. The benzoyl peroxide content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the milligrams of benzoyl peroxide that it is represented to contain. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1), except with respect to heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for erythromycin content and benzoyl peroxide content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The erythromycin used in making the batch: 5 packages, each containing approximately 100 milligrams.

(*b*) The batch: A minimum of 8 containers.

(b) *Tests and methods of assay*—(1) *Erythromycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Place an accurately weighed representative portion of the constituted product into a high-speed glass blender jar containing 0.5 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Benzoyl peroxide content.* Reconstitute the sample as directed in the labeling. Place an accurately weighted representative portion (about 2.5 grams) of the constituted product into a tared 250-milliliter glass-stoppered flask. Add 50 milliliters of glacial acetic acid and 20 milliliters of methylene chloride. Stopper and shake vigorously to disperse the gel. Add 1.0 milliliter phenylsulfide, swirl, stopper, and allow to stand at room temperature for 2 minutes. Purge the flask with nitrogen for 3 seconds. Add 5 milliliters for freshly prepared saturated sodium iodide solution, stopper, and swirl to mix. Let stand in the dark for 30 minutes. Add 50 milliliters of previously boiled and cooled distilled water and titrate the liberated iodine with 0.1*N* sodium thiosulfate, adding starch T.S. near the endpoint. Perform a blank determination and correct the sample titer. Each milliliter of 0.1*N* sodium thiosulfate is equivalent to 12.11 milligrams of benzoyl peroxide. Calculate the benzoyl peroxide content as follows:

$$\text{Percent benzoyl peroxide} = \frac{V_u \times \text{Normality of sodium thiosulfate} \times 12.11}{\text{Sample weight in grams}}$$

where:

V_u=Milliliters of sodium thiosulfate used in the titration of the sample minus the milliliters of sodium thiosulfate used in the titration of the sample blank.

[49 FR 47485, Dec. 5, 1984; 49 FR 49090, Dec. 18, 1984; 49 FR 49449, Dec. 20, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§ 452.510e Erythromycin topical gel.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin topical gel is erythromycin in a suitable and harmless gel. Each gram contains 20 milligrams of erythromycin. The erythromycin content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of erythromycin that it is represented to contain. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1), except with respect to heavy metals.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each request shall contain:

(i) Results of tests and assays on:

(A) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(B) The batch for erythromycin content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The erythromycin used in making the batch: 5 packages, each containing approximately 100 milligrams.

(B) The batch: A minimum of 8 containers.

(b) *Tests and methods of assay; erythromycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place approximately 1 gram, accurately weighed, of the product into a high-speed glass blender jar containing 200 milliliters of 0.5 percent (volume by volume) polysorbate 80 in 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

[53 FR 12415, Apr. 14, 1988; 53 FR 16837, May 11, 1988]

Subpart G [Reserved]

Subpart H—Rectal Dosage Forms

§ 452.710 Erythromycin suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin suppositories contain in each suppository 125 milligrams of erythromycin in a suitable and harmless base. The erythromycin content is satisfactory if it is not less than 90 percent nor more than 120 percent of the number of milligrams of erythromycin that it is represented to contain. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 452.10(a)(1), (i), (iii), (iv), (v), (vii), and (viii), except its moisture content is not more than 5.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 30 suppositories.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of suppositories for 3 to 5 minutes in a high-speed glass blender with 200 milliliters of methyl alcohol. Add 300 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), and blend again for 3 to 5 minutes. Remove an aliquot and dilute with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19149, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

Subpart I [Reserved]

Subpart J—Certain Other Dosage Forms

§ 452.910 Erythromycin for prescription compounding.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin for prescription compounding is the odorless, white to grayish-white or slightly yellow compound of a kind of erythromycin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It contains not less than 850 micrograms of erythromycin per milligram calculated on an anhydrous basis.

(ii) Its moisture content is not more than 10 percent.

(iii) Its pH is not less than 8.0 nor more than 10.5.

(iv) Its residue on ignition is not more than 2.0 percent.

(v) It gives a positive identity test for erythromycin.

(vi) It is crystalline.

(2) *Packaging.* The immediate container shall be a tight container as defined by the United States Pharmacopeia XXI. It shall be so sealed that the contents cannot be used without destroying such seal. Each such container shall contain 10 grams, 25 grams, or 100 grams of erythromycin.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its outside wrapper or container and on the immediate container the following:

(i) The statement ''Caution: Federal law prohibits dispensing without prescription.''

(ii) The statement ''Not sterile.''

(iii) The batch mark.

(iv) The number of micrograms of erythromycin activity in each milligram of erythromycin and the number of grams of erythromycin in the immediate container.

(v) The statement ''The potency of this drug cannot be assured for longer than 90 days after the container is first opened for compounding a prescription.''

(vi) The statements ''For use only in extemporaneous prescription compounding. Not for manufacturing use.''

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, residue on ignition, identity, and crystallinity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to obtain a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Dilute this solution further with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 3 to the reference concentration of 1.0 microgram of erythromycin base per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, except standardize the pH meter with pH 7.0 and pH 10.0 buffers and prepare the sample as follows: Dissolve 200 milligrams of sample in 5 milliliters of reagent grade methyl alcohol. Add 95 milliliters of water and mix. Record the pH when an equilibrium value has been reached.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Identity test.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[51 FR 35213, Oct. 2, 1986, as amended at 55 FR 11584, Mar. 29, 1990; 55 FR 25392, June 21, 1990]

PART 453—LINCOMYCIN ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
453.20 Clindamycin hydrochloride hydrate.
453.21 Clindamycin palmitate hydrochloride.
453.22 Clindamycin phosphate.
453.22a Sterile clindamycin phosphate.
453.30 Lincomycin hydrochloride monohydrate.
453.30a Sterile lincomycin hydrochloride monohydrate.

Subpart B—Oral Dosage Forms

453.120 Clindamycin hydrochloride hydrate capsules.
453.121 Clindamycin palmitate hydrochloride oral dosage forms.
453.121a Clindamycin palmitate hydrochloride for oral suspension.
453.121b Clindamycin palmitate hydrochloride for oral solution.
453.130 Lincomycin hydrochloride oral dosage forms.
453.130a Lincomycin hydrochloride monohydrate capsules.
453.130b Lincomycin hydrochloride syrup.

Subpart C—Injectable Dosage Forms

453.222 Clindamycin phosphate injection.
453.230 Lincomycin hydrochloride injection.

Subparts D–E [Reserved]

Subpart F—Dermatologic Dosage Forms

453.522 Clindamycin phosphate dermatologic dosage forms.
453.522a Clindamycin phosphate topical solution.
453.522b Clindamycin phosphate gel.
453.522c Clindamycin phosphate lotion.
453.522d Clindamycin phosphate vaginal cream.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§453.20 Clindamycin hydrochloride hydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate is the hydrated hydrochloride salt of clindamycin. It is so purified and dried that:

(i) Its clindamycin content is not less than 800 micrograms of clindamycin per milligram.

(ii) Its microbiological activity is not less than 800 micrograms of clindamycin per milligram.

(iii) [Reserved]

(iv) Its moisture content is not less than 3.0 percent and not more than 6.0 percent.

(v) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(vi) It is crystalline.

(vii) It passes the identity test for clindamycin hydrochloride hydrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clindamycin content* (*vapor phase chromatography*). Proceed as directed in §436.302 of this chapter.

(2) *Microbiological activity* (*microbiological agar diffusion assay.*) Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(3) [Reserved]

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in §436.203 of this chapter.

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

[39 FR 19161, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 453.21 Clindamycin palmitate hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride is the white to off-white amorphous powder of the hydrochloride salt of the palmitic acid ester of clindamycin. It is freely soluble in water, ethanol, chloroform, and ether. It is so purified and dried that:

(i) It contains not less than 540 micrograms of clindamycin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 3.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.8 and not more than 3.8.

(v) It passes the identity test for clindamycin palmitate hydrochloride.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, moisture, pH, and identity.

(ii) Samples required: 10 packages, nine containing not less than 300 milligrams and one package containing not less than 2 grams.

(b) *Tests and methods of assay*—(1) *Clindamycin content.* Proceed as directed in § 436.303 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

[39 FR 19161, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 453.22 Clindamycin phosphate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate is a water-soluble ester of clindamycin and phosphoric acid. It occurs as a white to off-white powder. It is so purified and dried that:

(i) Its clindamycin content is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(ii) Its microbiological activity is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(iii) [Reserved]

(iv) Its moisture content is not more than 6.0 percent.

(v) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 4.5.

(vi) It is crystalline.

(vii) It passes the identity test for clindamycin phosphate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, nine containing approximately 300 milligrams and one containing 1.5 grams.

(b) *Tests and methods of assay*—(1) *Clindamycin content (vapor phase chromatography).* Proceed as directed in § 436.304 of this chapter.

(2) *Microbiological activity (microbiological agar diffusion assay).* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters of chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of

activity[1] and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C ±2° C for 2.5 hours. After the 2.5-hours hydrolysis, allow the solution to cool. Further dilute an aliquot of the solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(3) [Reserved]

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in §436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section, except dry the sample for 2 hours at 100° C and allow to equilibrate with the atmosphere for 1 hour.

[46 FR 2996, Jan. 13, 1981, as amended at 50 FR 19921, May 13, 1985]

§453.22a Sterile clindamycin phosphate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile clindamycin phosphate is a water-soluble ester of clindamycin and phosphoric acid. It occurs as a white to off-white powder. It is so purified and dried that:

(i) Its clindamycin content is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(ii) Its microbiological activity is not less than 758 micrograms of clindamycin per milligram calculated on an anhydrous basis.

(iii) It is sterile.

(iv) It is nonpyrogenic.

(v) [Reserved]

(vi) It contains no depressor substances.

(vii) Its moisture content is not more than 6 percent.

(viii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 4.5.

(ix) It is crystalline.

(x) It passes the identity test for clindamycin phosphate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for clindamycin content, microbiological activity, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, nine containing approximately 300 milligrams and one containing 1.5 grams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clindamycin content* (*vapor phase chromatography*). Proceed as directed in §436.304 of this chapter.

(2) *Microbiological activity* (*microbiological agar diffusion assay*). Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters of chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity[1] and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C.±2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool. Further dilute an aliquot of the solution with

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 24 milligrams of clindamycin per milliliter.

(5) [Reserved]

(6) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(7) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(8) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(9) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(10) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section, except dry the sample for 2 hours at 100° C. and allow to equilibrate with the atmosphere for 1 hour.

[39 FR 19161, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

§ 453.30 Lincomycin hydrochloride monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate is the monohydrated hydrochloride salt of lincomycin. It is freely soluble in water and soluble in acetone and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 790 micrograms of lincomycin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.0 percent and is not more than 6.0 percent.

(iv) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(v) Its specific rotation in an aqueous solution at 25° C. is not less than +135° and not more than +150°.

(vi) It passes the infrared identity test.

(vii) Its content of lincomycin B is not more than 5 percent.

(viii) It passes the identity test if the elution pattern of the lincomycin sample compares quantitatively to that of the lincomycin working standard under identical conditions of gas liquid chromatography.

(ix) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the gas liquid chromatography assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.5 microgram of lincomycin per milliliter (estimated).

(ii) *Gas liquid chromatography assay.* Proceed as directed in § 436.306 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(5) *Specific rotation.* Accurately weigh 500 milligrams of lincomycin hydrochloride monohydrate in a 25-milliliter, glass stoppered volumetric flask and fill to volume with distilled water. Proceed as directed in § 436.210 of this

chapter, using a 2.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(6) *Infrared absorption spectrum.* Proceed as directed in §436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(7) *Lincomycin B content.* Proceed as directed in §436.306 of this chapter.

(8) *Identity.* Proceed as described in §436.306 of this chapter.

(9) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19161, May 30, 1974, as amended at 46 FR 3839, Jan. 16, 1981; 50 FR 19921, May 13, 1985]

§453.30a Sterile lincomycin hydrochloride monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate is the monohydrated hydrochloride salt of lincomycin. It is freely soluble in water and soluble in acetone and dimethylformamide. It is so purified and dried that:

(i) Its potency is not less than 790 micrograms of lincomycin per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 3.0 percent and not more than 6.0 percent.

(vii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 5.5.

(viii) Its specific rotation in an aqueous solution at 25° C. is not less than +135° and not more than +150°.

(ix) It passes the infrared identity test.

(x) Its content of lincomycin B is not more than 5 percent.

(xi) It passes the identity test if the elution pattern of the lincomycin sample compares quantitatively to that of the lincomycin working standard under identical conditions of gas liquid chromatography.

(xii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain.

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the gas liquid chromatography assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.5 microgram of lincomycin per milliliter (estimated).

(ii) *Gas liquid chromatography assay.* Proceed as directed in §436.306 of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 0.5 milligram of lincomycin per milliliter.

(5) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(6) *Moisture.* Proceed as directed in §436.201 of this chapter.

(7) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(8) *Specific rotation.* Accurately weigh 500 milligrams of lincomycin hydrochloride monohydrate in a 25 milliliter, glass-stoppered volumetric flask and fill to lincomycin B content, crystallinity, and volume with distilled water. Proceed as directed in § 436.210, using a 2.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(9) *Infrared absorption spectrum.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(2) of that section.

(10) *Lincomycin B content.* Proceed as directed in § 436.306 of this chapter.

(11) *Identity.* Proceed as directed in § 436.306 of this chapter.

(12) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19161, May 30, 1974, as amended at 46 FR 3839, Jan. 16, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 453.120 Clindamycin hydrochloride hydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate capsules are composed of clindamycin hydrochloride hydrate and one or more suitable and harmless diluents and lubricants. Each capsule contains clindamycin hydrochloride hydrate equivalent to 75, 150, or 300 milligrams of clindamycin. Its content of clindamycin is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin that it is represented to contain. The moisture content is not more than 7.0 percent. The clindamycin hydrochloride hydrate used conforms to the standards prescribed by § 453.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin hydrochloride hydrate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for clindamycin content and moisture.

(ii) Samples required:

(*a*) The clindamycin hydrochloride hydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Clindamycin content* (*vapor phase chromatography*). Proceed as directed in § 436.302 of this chapter, except:

(i) *Preparation of clindamycin sample and working standard solutions.* Accurately weigh a portion of the clindamycin working standard equivalent to about 45 milligrams of clindamycin and transfer to a 15-milliliter glass-stoppered centrifuge tube. Empty 20 capsules, collecting the contents quantitatively. Weigh the powder and determine the average capsule fill weight. Mix the powder and accurately weigh a portion containing the equivalent of about 45 milligrams of clindamycin into a second 15-milliliter glass-stoppered centrifuge tube. Add 3 milliliters of 1 percent sodium carbonate solution and 3 milliliters of chloroform to each tube. Shake the solution vigorously and then centrifuge. Remove the top aqueous layer and add approximately 1 gram of anhydrous sodium sulfate to dry the chloroform layer. Place a 1-milliliter aliquot of the chloroform solution into a 15-milliliter centrifuge tube, add 1 milliliter of internal standard and 0.6 milliliter of acetic anhydride. Agitate the vials to insure complete mixing of the liquids.

(ii) *Calculations.* Calculate the clindamycin content of the capsules as follows:

$$\text{Milligrams of clindamycin per capsule} = \frac{R_u \times W_s \times f \times W_a}{R_s \times W_u}$$

where:

R_u=Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard)/Area of internal standard peak;

R_s=Area of the clindamycin standard peak/Area of internal standard peak;

W_s=Weight of clindamycin working standard in milligrams;

W_u=Sample weight in milligrams;

f=Potency of clindamycin working standard in milligrams of clindamycin per milligram;

W_a=Average capsule fill weight in milligrams,

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19161, May 30, 1974, as amended at 50 FR 19921, May 13, 1985; 54 FR 41824, Oct. 12, 1989; 54 FR 43384, Oct. 24, 1989]

§453.121 Clindamycin palmitate hydrochloride oral dosage forms.

§453.121a Clindamycin palmitate hydrochloride for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride for oral suspension is composed of clindamycin palmitate hydrochloride with one or more suitable and harmless diluents, buffer substances, colorings, and flavorings. When reconstituted as directed in the labeling, using the accompanying diluent when provided, each milliliter contains clindamycin palmitate hydrochloride equivalent to 15 milligrams of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin that it is represented to contain. The moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 5.0. The clindamycin palmitate hydrochloride used conforms to the standards prescribed by §453.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin palmitate hydrochloride used in making the batch for clindamycin content, moisture, pH, and identity.

(*b*) The batch for clindamycin content, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin palmitate hydrochloride used in making the batch: 10 packages, nine containing not less than 300 milligrams, and one containing not less than 2 grams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content.* Proceed as directed in §436.303 of this chapter, except:

(i) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh about 130 milligrams of the clindamycin palmitate hydrochloride working standard and transfer to a 25-milliliter volumetric flask. Add 5 milliliters of distilled water. Reconstitute the clindamycin palmitate hydrochloride for oral suspension as directed in the labeling, using the accompanying diluent when provided, and transfer exactly 5.0 milliliters to a 25-milliliter volumetric flask. Add exactly 5.0 milliliters of internal standard and 1 milliliter of 30 percent sodium carbonate to each flask. Shake both flasks mechanically for 5 minutes. Transfer the contents of each flask to separate 15-milliliter glass-stoppered centrifuge tubes and centrifuge. Remove the top aqueous layer by suction and transfer exactly 1.0 milliliter of the chloroform layer to separate glass-stoppered, conical, 15-milliliter centrifuge tubes. Add 1 milliliter of pyridine and 0.5 milliliter of acetic anhydride. Agitate the tubes to insure complete mixing of the liquids. Proceed as directed in §436.303(e) of this chapter.

(ii) Calculations: Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{R_u \times W_s \times f}{R_s \times V}$$

where:

R_u=Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard)/Area of internal standard peak;

R_s=Area of the clindamycin palmitate hydrochloride standard peak/Area of internal standard peak;

W_s=Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

V=Volume of reconstituted sample in milliliters;

f=Milligrams of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 19161, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 453.121b Clindamycin palmitate hydrochloride for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin palmitate hydrochloride for oral solution is composed of clindamycin palmitate hydrochloride with one or more suitable and harmless diluents, buffer substances, colorings, flavorings, and preservatives. When reconstituted as directed in the labeling, each milliliter contains clindamycin palmitate hydrochloride equivalent to 15 milligrams of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of clindamycin that it is represented to contain. The moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 5.0. The clindamycin palmitate hydrochloride used conforms to the standards prescribed by § 453.21(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin palmitate hydrochloride used in making the batch for clindamycin content, moisture, pH, and identity.

(*b*) The batch for clindamycin content, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin palmitate hydrochloride used in making the batch: 10 packages, nine containing not less than 300 milligrams, and one containing not less than 2 grams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content.* Proceed as directed in § 436.303 of this chapter, except:

(i) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh about 130 milligrams of the clindamycin palmitate hydrochloride working standard and transfer to a 25-milliliter volumetric flask. Add 5 milliliters of distilled water. Reconstitute the clindamycin palmitate hydrochloride for oral solution as directed in the labeling and transfer exactly 5.0 milliliters to a 25-milliliter volumetric flask. Add exactly 5.0 milliliters of internal standard and 1 milliliter of 30-percent sodium carbonate to each flask. Shake both flasks mechanically for 5 minutes. Transfer the contents of each flask to separate 15-milliliter glass-stoppered centrifuge tubes and centrifuge. Remove the top aqueous layer by suction and transfer exactly 1.0 milliliter of the chloroform layer to separate glass-stoppered, conical, 15-milliliter centrifuge tubes. Add 1 milliliter of pyridine and 0.5 milliliter of acetic anhydride. Agitate the tubes to insure complete mixing of the liquids. Proceed as directed in § 436.303(e) of this subchapter.

(ii) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{R_u \times W_s \times f}{R_s \times V}$$

where:

R_u=Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard);/Area of internal standard peak;

R_s=Area of the clindamycin palmitate hydrochloride standard peak;/Area of internal standard peak;

W_s=Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

V=Volume of reconstituted sample in milliliters;

f=Milligrams of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

(2) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(3) *pH.* Proceed as directed in §436.202 of this subchapter, using the drug reconstituted as directed in the labeling.

[39 FR 19161, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§453.130 Lincomycin hydrochloride oral dosage forms.

§453.130a Lincomycin hydrochloride monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate capsules are composed of lincomycin hydrochloride monohydrate and suitable diluents, enclosed in a gelatin capsule. Each capsule contains 250 milligrams of lincomycin or 500 milligrams of lincomycin. The lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. Its moisture content is not more than 7.0 percent. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by §453.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, moisture, pH, specific rotation, infrared absorption spectrum, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the gas liquid chromatography assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high–speed glass blender jar with sufficient sterile distilled water to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.5 microgram of lincomycin per milliliter (estimated).

(ii) *Gas liquid chromatography assay.* Proceed as directed in §436.306 of this chapter, except prepare the sample for assay as follows: Place the contents of 5 capsules in a 100-milliliter volumetric flask and add about 60 milliliters of methanol. Place on a steam bath and allow to boil gently for 5 minutes. Remove from the steam bath, add more methanol, and adjust to mark after cooling to ambient temperature. Dilute an aliquot equivalent to 50 milligrams of lincomycin to 25 milliliters with methanol. Transfer 2 milliliters to a centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content of the capsules as follows:

$$\text{Lincomycin content in milligrams per capsule} = \frac{R_u \times W_s \times d \times f}{R_s \times N}$$

where:

R_u=Area of lincomycin sample peak/Area of internal standard;

R_s=Area of lincomycin standard peak/Area of internal standard;

W_s=Weight of lincomycin working standard in milligrams;

d=Dilution factor;

f=Potency of lincomycin working standard in milligrams of lincomycin per milligram;

N=Number of capsules used.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[39 FR 19161, May 30, 1974, as amended at 46 FR 3839, Jan. 16, 1981; 50 FR 19921, May 13, 1985]

§453.130b Lincomycin hydrochloride syrup.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride syrup is a syrup containing lincomycin hydrochloride monohydrate, one or

more suitable preservatives, flavorings, sweetening agents, colorings, and purified water. Each milliliter contains lincomycin hydrochloride equivalent to either 25 milligrams or 50 milligrams of lincomycin. Its lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. The pH is not less than 3 and not more than 5.5. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30(a)(1) (i), (iv), (v), (vi), (vii), (viii), and (ix).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, pH, specific rotation, infrared absorption spectrum, lincomycin B content, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the gas liquid chromatography assay shall be conclusive.

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Remove an accurately measured representative sample with a suitable hypodermic needle and syringe. Place into a high-speed glass blender jar with sufficient sterile distilled water to give a total volume of 500 milliliters. Blend for 3 to 5 minutes. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.5 microgram of lincomycin per milliliter (estimated).

(ii) *Gas liquid chromatography assay*. Proceed as directed in § 436.306 of this chapter, except prepare the sample for assay by either of the following methods:

(*a*) Place an aliquot of syrup, containing the equivalent of 250 milligrams of lincomycin into a 50-milliliter volumetric flask and add 30 milliliters of absolute ethanol. Place on a steam bath and boil gently for 5 minutes. Remove from the steam bath and cool. Add ethanol to prior volume level and let stand overnight. Adjust to mark, shake well, and transfer a 5-milliliter aliquot into a 25-milliliter volumetric flask and make to mark with methanol. Place 4 milliliters of this solution in a 15-milliliter centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter} = \frac{R_u \times W_s \times d \times f}{R_s \times M}$$

where:

R_u=Area of lincomycin sample peak/Area of internal standard;

R_s=Area of lincomycin standard peak/Area of internal standard;

W_s=Weight of lincomycin working standard in milligrams;

d=Dilution factor;

f=Potency of lincomycin working standard in milligrams of lincomycin per milligram;

M=Milliliters of syrup used.

(*b*) Treat the lincomycin working standard and sample in a similar manner, except lyophilize an aliquot of the sample containing the equivalent of 50 milligrams of lincomycin. To approximately 50 milligrams of the standard, accurately weighed, and to the dried residue of the sample, add 5 milliliters of dry pyridine which contains 10 milligrams of tetraphenylcyclopentadienone per milliliter. Warm on a hot plate for 5 minutes to attain complete solution. Remove from the hot plate and add 5 milliliters of hexamethyldisilazane and 2 milliliters of trimethylchlorosilane. Shake mechanically for 60 minutes, then centrifuge for 15 minutes. Inject 2 microliters of the supernate into the chromatograph. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter of syrup} = \frac{R_u \times W_s \times f}{R_s \times M}$$

where:

R_u=Area of lincomycin sample peak/Area of internal standard;

R_s=Area of lincomycin standard peak/Area of internal standard;

W_s=Weight of lincomycin working standard in milligrams;

f=Potency of lincomycin working standard in milligrams of lincomycin per milligram;

M=Milliliters of syrup used.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19161, May 30, 1974, as amended at 46 FR 3840, Jan. 16, 1981; 50 FR 19921, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 453.222 Clindamycin phosphate injection.

(a)(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate injection is an aqueous solution of clindamycin phosphate with one or more suitable and harmless preservatives, sequestering agents, or tonicity agents. It may be frozen. Its clindamycin phosphate content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of clindamycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its pH is not less than 5.5 and not more than 7. The clindamycin phosphate used conforms to the standards prescribed by § 453.22a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for clindamycin content, sterility, pyrogens, depressor substances, and pH.

(ii) Samples required:

(*a*) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) Clindamycin content. Use any of the following methods. However, the results obtained from the high performance liquid chromatographic assay shall be conclusive.

(i) *Vapor phase chromatography.* Proceed as directed in § 436.304 of this chapter, except prepare the sample for assay as follows: Shake the sample and dilute a portion with pH 9.0 borate buffer to obtain a solution containing the equivalent of approximately 0.4 milligrams of clindamycin per milliliter. Place 25 milliliters of this solution into a 50-milliliter stoppered centrifuge tube. Add 10 milliliters of chloroform. Shake vigorously for 15 minutes and centrifuge. There should be no emulsion present after centrifugation. Transfer 20 milliliters of the aqueous phase from the tube into a 35-milliliter stoppered centrifuge tube. Add to the tube a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity[1] and allow to stand until the phosphatase has dissolved completely. Place the centrifuge tube into a water bath at 37°C±2°C for 2.5 hours. After the 2.5-hours hydrolysis, allow the solution to cool.

(ii) *High performance liquid chromatographic assay.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nonometers, a 25-centimeter long × 4.6 millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase octysilane hydrocarbon bonded

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in § 436.304 of this chapter.

silica packing material, a flow rate of about 1.0 milliliter per minute, and a known injection volume of between 10 and 20 microliters. The retention time of clindamycin phosphate, and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(*a*) *Reagents*—(*1*) *0.1M Potassium phosphate monobasic buffer*. Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(*2*) *Mobile phase*. Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(*b*) *Preparation of working standard, sample, and resolution test solutions*—(*1*) *Working standard solution*. Dissolve an accurately weighed portion of the clindamycin phosphate working standard with sufficient mobile phase (prepared as directed in paragraph (b)(1)(ii)(*a*)(*2*) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(*2*) *Sample solution*. Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient mobile phase (prepared as directed in paragraph (b)(1)(ii)(*a*)(*2*) of this section) to obtain a solution containing 200 micrograms of clindamycin per milliliter (estimated).

(*3*) *Resolution test solution*. Place 15 milligrams each of clindamycin phosphate, and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute with mobile phase and mix well. Use this solution to determine the resolution factor.

(*c*) *System suitability requirements*—(*1*) *Asymmetry factor*. Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a = Horizontal distance from point of ascent to point of maximum peak height; and

b = Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not more than 1.3.

(*2*) *Efficiency of the column*. From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;

n = Number of theoretical plates; and

d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(*3*) *Resolution factor*. The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin hydrochloride in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(*4*) *Coefficient of variation* (*relative standard deviation*). The coefficient of variation (S_R in percent) of 5 replicate injections of the working standard solution (prepared as directed in paragraph (b)(1)(ii)(*b*)(*1*) of this section is satisfactory if it is not more than 2.5 percent.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(*d*) *Calculations*. Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin

phosphate working standard;

P_s = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing the equivalent of 24 milligrams of clindamycin per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[39 FR 19161, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985; 54 FR 43289, Oct. 24, 1989; 55 FR 5842, Feb. 20, 1990]

§ 453.230 Lincomycin hydrochloride injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride injection is an aqueous solution of lincomycin hydrochloride monohydrate containing benzyl alcohol as a preservative. Each immediate container contains either 1, 2, or 10 milliliters of a solution containing, in each milliliter, 300 milligrams of lincomycin, and 9 milligrams of benzyl alcohol. The lincomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its pH is not less than 3.0 and not more than 5.5. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by § 453.30a(a)(1) (i), (vi), (vii), (viii), (ix), (x), and (xi).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. If each immediate container contains only 1 milliliter of the drug, the labeling shall include the statement ''For pediatric use''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, moisture, pH, specific rotation, infrared absorption spectrum, lincomycin B content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, and pH.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the gas liquid chromatography assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Place the portion, thus obtained, into a suitably-sized volumetric flask and dilute to volume with sterile distilled water. Remove an aliquot and further dilute with sterile distilled water to the reference concentration of 0.5 microgram of lincomycin per milliliter (estimated).

(ii) *Gas liquid chromatography assay.* Proceed as directed in § 436.306 of this chapter, except prepare the sample for assay as follows: Dilute the equivalent of 300 milligrams of lincomycin to 50 milliliters with methanol and shake. Transfer a 3-milliliter aliquot to a 10-milliliter volumetric flask and make to

mark with methanol. Place a 2-milliliter aliquot into a 15-milliliter centrifuge tube and evaporate to dryness on a steam bath with a stream of dry air. Dissolve the residue in 1 milliliter of dry pyridine. Calculate the lincomycin content as follows:

$$\text{Lincomycin content in milligrams per milliliter} = \frac{R_u \times W_s \times d \times f}{R_s \times \text{number of milliliters of sample}}$$

where:

$$R_u = \frac{\text{Area of lincomycin sample peak}}{\text{Area of internal standard}}$$

$$R_s = \frac{\text{Area of lincomycin standard peak}}{\text{Area of internal standard}}$$

W_s=Weight of lincomycin working standard in milligrams;
d=Dilution factor;
f=Potency of lincomycin working standard in milligrams of lincomycin per milligram

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 milligram of lincomycin per milliliter.

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[39 FR 19161, May 30, 1974, as amended at 46 FR 3841, Jan. 16, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

Subparts D–E [Reserved]

Subpart F—Dermatologic Dosage Forms

§ 453.522 Clindamycin phosphate dermatologic dosage forms.

§ 453.522a Clindamycin phosphate topical solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate is a solution of clindamycin phosphate in a suitable and harmless vehicle. Each milliliter contains 10 milligrams of clindamycin activity. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 4.0 and not more than 7.0. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for clindamycin content and pH.

(ii) Samples required:

(*a*) The clindamycin phosphate used in making the batch: 6 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content* (*vapor phase chromatography*). Proceed as directed in § 436.304 of this chapter, except prepare the sample for assay and calculate the clindamycin content as follows:

(i) *Preparation of the sample.* Accurately transfer a volume of sample equivalent to approximately 20 milligrams of clindamycin activity to a 50-milliliter volumetric flask. Evaporate the sample to near dryness under a stream of nitrogen. Dilute to 50 milliliters with pH 9.0 borate buffer and mix well. Place 25.0 milliliters of this solution into a 50-milliliter stoppered centrifuge tube. Add 10 milliliters of chloroform. Shake vigorously for 15 minutes and centrifuge to obtain adequate phase separation of the chloroform and aqueous phase. Transfer 20 milliliters of the aqueous phase from the tube into a 35-milliliter stoppered centrifuge tube. Add to the tube a weighed

amount of intestinal alkaline phosphatase equivalent to 50 units of activity[1] and allow to stand until the phosphatase has dissolved completely. Place the centrifuge tube into a water bath at 37° C ± 2° C for 2.5 hours. After the 2.5-hours hydrolysis, allow the solution to cool.

(ii) *Calculations.* Calculate the clindamycin content as follows:

Clindamycin content per milliliter=$(R_u \times W \times_s \times d \times f)/(R_s \times V)$

where:

R_u=Area of clindamycin sample peak/Area of internal standard;

R_s=Area of clindamycin standard peak/Area of internal standard;

W_s=Weight of clindamycin working standard in milligrams;

d=Dilution factor;

f=Potency of clindamycin working standard in milligrams of clindamycin per milligram;

V=Volume of sample in milliliters.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[46 FR 2997, Jan. 13, 1981. Redesignated at 54 FR 38224, Sept. 15, 1989]

§ 453.522b Clindamycin phosphate gel.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate gel contains clindamycin phosphate in a suitable and harmless vehicle. Each gram contains clindamycin phosphate equivalent to 10 milligrams of clindamycin activity. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(B) The batch for clindamycin content, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content (High performance liquid chromatographic assay).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, a 25-centimeter long x 4.6-millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase octysilane hydrocarbon bonded silica packing material, a flow rate of about 1.0 milliliter per miunute, and a known injection volume of between 10 and 20 microliters. The retention time of clindamycin phosphate, and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standards and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Potasium phosphate monobasic buffer.* Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(B) *Mobile phase.* Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A)

[1] Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in § 436.304 of this chapter.

Working standard solution. Dissolve an accurately weighed portion of the clindamycin phosphate working standard with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(B) *Sample solution.* Accurately weigh and transfer approximately 2.0 grams of the sample into a 100-milliliter volumetric flask. Dilute to volume with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) and shake vigorously for 30 minutes. Centrifuge a portion of the solution and if necessary filter a few milliliters of the centrifuged solution through a 2-micron millipore filter, type BS.

(C) *Resolution test solution.* Place 15 milligrams each of clindamycin phosphate and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute to volume with mobile phase and mix well. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a = Horizontal distance from point of ascent to point of maximum peak height; and

b = Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not more than 1.3.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;

n = Number of theoretical plates; and

d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor.* The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections of the working standard solution (prepared as directed in paragraph (b)(1)(ii)(A) of this section is satisfactory if it is not more than 2.5 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;

P_s = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(A) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted gel.

(B) *Identity.* The high-performance liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[54 FR 38224, Sept. 15, 1989]

§ 453.522c Clindamycin phosphate lotion.

(a) *Requirements for certification*—(1) *Standards for identity, strength, quality, and purity.* Clindamycin phosphate lotion contains clindamycin phosphate in a suitable and harmless lotion vehicle, with one or more suitable and harmless emollients, buffers, and dispersants. Each milliliter contains clindamycin phosphate equivalent to 10 milligrams

of clindamycin. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(B) The batch for clindamycin content, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content (high performance liquid chromatographic assay).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an untraviolet detection system operating at a wavelength of 210 nanometers, a 25-centimeter long x 4.6 millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reversed phase octysilane hydrocarbon bonded silica packing material, a flow rate of about 1.08 milliliter per minute, and a known injection volume of between 10 and 20 microliters. The retention time of clindamycin phosphate and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *O.1M Potassium phosphate monobasic buffer.* Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(B) *Mobile phase.* Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighted portion of the clindamycin phosphate working standard with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(B) *Sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin per milliliter (estimated).

(C) *Resolution test solution.* Dissolve 30 milligrams of clindamycin phosphate in 25 milliliters of mobile phase. Dissolve 30 milligrams of clindamycin hydrochloride in 25 milliliters of mobile phase. Combine both solutions in a 50-milliliter volumetric flask and shake or use a vortex shaker to assure mixture of both solutions. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the base line as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not more than 1.3.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;
n=Number of theoretical plates; and
d_p=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor.* The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$in percent) of 5 replicate injections of the working standard solution (prepared as directed in paragraph (b)(1)(ii)(A) of this section is satisfactory if it is not more than 2.5 percent.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;
P_s=Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and
d=Dilution factor of the sample.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted lotion.

(3) *Identity.* The high-performance liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[54 FR 40655, Oct. 3, 1989]

§ 453.522d Clindamycin phosphate vaginal cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin phosphate vaginal cream contains clindamycin phosphate in a suitable and harmless cream vehicle. Each gram contains clindamycin phosphate equivalent to 20 milligrams of clindamycin activity. Its clindamycin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of clindamycin that it is represented to contain. Its pH is not less than 3.0 and not more than 6.0. It passes the identity test. The clindamycin phosphate used conforms to the standards prescribed by § 453.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The clindamycin phosphate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(B) The batch for clindamycin content, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The clindamycin phosphate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: a minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Clindamycin content (high performance liquid chromatography assay).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, a 25-centimeter long x 4.6 millimeter ID column packed with microparticulate (5 to 10 micrometers in diameter) reverse

phase octylsilane hydrocarbon bonded silica packing material, a flow rate of 1.0 milliliter per minute, and a known injection volume of 20 microliters. The retention time of clindamycin phosphate, and clindamycin are approximately 6 and 9 minutes, respectively. Reagents, working standards and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M Potassium phosphate monobasic buffer*. Dissolve 13.61 grams of potassium phosphate monobasic in 775 milliliters of water. Adjust the pH to 2.5 with phosphoric acid. Further dilute with water to a volume of 1,000 milliliters.

(B) *Mobile phase*. Mix 225 milliliters of acetonitrile and 775 milliliters of 0.1*M* potassium phosphate, pH 2.5 buffer (225:775). Filter through a suitable filter capable of removing particulate matter greater than 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution*. Dissolve an accurately weighed portion of the clindamycin phosphate working standard in sufficient mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section) to obtain a solution containing 200 micrograms of clindamycin activity per milliliter.

(B) *Sample solutions*. Accurately weigh and transfer approximately 1.0 gram of the sample into a 125-milliliter Erlenmeyer flask. Add 100.0 milliliters of mobile phase (prepared as directed in paragraph (b)(1)(i)(B) of this section), accurately measured, and 8 to 10 glass beads (4 to 5 millimeters). Close the flask securely using a plastic stopper and shake vigorously by mechanical means for 1 hour at 50 °C. Cool in an ice bath for approximately 20 minutes. Centrifuge a portion of the mixture. Use the lower cloudy solution for chromatographic analysis. Filter a few milliliters of the centrifuged solution through an appropriate 2 micron filter.

(C) *Resolution test solution*. Place 15 milligrams each of clindamycin phosphate and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute to volume with mobile phase and mix well. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor*. Calculate the asymmetry factor (A_S), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a = Horizontal distance from point of ascent to point of maximum peak height; and

b = Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 1.0 and not more than 1.3.

(B) *Efficiency of the column*. From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height (h_r) as follows;

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L = Length of the column in centimeters;

n = Number of theoretical plates; and

d_p = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 15.

(C) *Resolution factor*. The resolution factor (R) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent) of 5 replicate injections of the working standard solution is satisfactory if it is not more than 2.5 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculation*. Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;

P_s = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted cream.

(3) *Identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[60 FR 49508, Sept. 26, 1995]

PART 455—CERTAIN OTHER ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.
455.4 Aztreonam.
455.4a Sterile aztreonam.
455.10 Chloramphenicol.
455.10a Sterile chloramphenicol.
455.11 Chloramphenicol palmitate.
455.12a Sterile chloramphenicol sodium succinate.
455.15 Clavulanate potassium.
455.15a Sterile clavulanate potassium.
455.20 Cycloserine.
455.40 Mupirocin.
455.50 Calcium novobiocin.
455.51 Sodium novobiocin.
455.51a Sterile sodium novobiocin.
455.70 Rifampin.
455.80a Sterile spectinomycin hydrochloride.
455.82a Sterile sulbactam sodium.
455.85 Vancomycin hydrochloride.
455.85a Sterile vancomycin hydrochloride.
455.86 Vancomycin.
455.88 Rifabutin.
455.90a Sterile vidarabine monohydrate.

Subpart B—Oral Dosage Forms

455.110 Chloramphenicol capsules.
455.111 Chloramphenicol palmitate oral suspension.
455.120 Cycloserine capsules.
455.150 Calcium novobiocin oral suspension.
455.151 Sodium novobiocin oral dosage forms.
455.151a Sodium novobiocin tablets.
455.151b Sodium novobiocin capsules.
455.170 Rifampin oral dosage forms.
455.170a Rifampin capsules.
455.170b Rifampin-isoniazid capsules.
455.185 Vancomycin hydrochloride oral dosage forms.
455.185a Vancomycin hydrochloride for oral solution.
455.185b Vancomycin hydrochloride capsules.
455.188 Rifabutin capsules.

Subpart C—Injectable Dosage Forms

455.204 Aztreonam injectable dosage forms.
455.204a Aztreonam for injection.
455.204b Aztreonam injection.
455.210 Chloramphenicol injection.
455.212 Sterile chloramphenicol sodium succinate.
455.230 Moxalactam disodium for injection.
455.251 Sodium novobiocin for injection.
455.270 Rifampin for injection.
455.280a Sterile spectinomycin hydrochloride.
455.285 Vancomycin hydrochloride injectable dosage forms.
455.285a Sterile vancomycin hydrochloride.
455.285b Vancomycin hydrochloride for injection.
455.285c Vancomycin hydrochloride injection.
455.290 Vidarabine monohydrate for infusion.

Subpart D—Ophthalmic Dosage Forms

455.310 Chloramphenicol ophthalmic dosage forms.
455.310a Chloramphenicol ophthalmic solution.
455.310b Chloramphenicol for ophthalmic solution.
455.310c Chloramphenicol ointment (chloramphenicol cream).
455.310d Chloramphenicol-polymyxin ointment.
455.310e Chloramphenicol-hydrocortisone acetate for ophthalmic suspension.
455.390 Vidarabine monohydrate ophthalmic ointment.

Subpart E—Otic Dosage Forms

455.410 Chloramphenicol otic.

Subpart F—Dermatologic Dosage Forms

455.510 Chloramphenicol dermatologic dosage forms.
455.510a Chloramphenicol ointment (chloramphenicol cream).
455.510b [Reserved]
455.510c Chloramphenicol-polymyxin ointment.
455.510d Fibrinolysin and desoxyribonuclease, combined (bovine) with chloramphenicol ointment.
455.540 Mupirocin ointment.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19166, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§ 455.4 Aztreonam.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam is a practically odorless, white to slightly off-white fine powder. It is sparingly soluble in water of pH 2, and is very soluble at pH values above 4. Its solubility is slight to very slight in polar organic solvents such as methanol and ethanol and it is insoluble in nonpolar solvents such as hexane and heptane. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of aztreonam per milligram on an ''as is'' basis.

(ii) Its moisture content is not more than 2.0 percent.

(iii) Its residue on ignition is not more than 0.1 percent.

(iv) Its heavy metals content is not more than 30 parts per million.

(v) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requirements for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, residue on ignition, heavy metals, and identify.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research; 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 455.4a(b)(1).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(4) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[54 FR 40385, Oct. 2, 1989]

§ 455.4a Sterile aztreonam.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam is a practically odorless, white to slightly off-white fine powder. It is sparingly soluble in water of pH 2, and is very soluble at pH values above 4. Its solubility is slight to very slight in polar organic solvents such as methanol and ethanol and it is insoluble in non-polar solvents such as hexane and heptane. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of aztreonam per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 2.0 percent.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) Its heavy metals content is not more than 30 parts per million.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requirements for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.361 of this chapter, except in lieu of the guard column described in paragraph (a)(4) of that section, use a 5- to 10-centimeter precolumn having an inside diameter of 2 millimeters and packed with octadecyl silane chemically bonded to silica gel of a controlled surface porosity

that has been bonded to a solid spherical core (U.S.P. designation L–2) 30 micrograms to 50 micrograms in diameter; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 270 nanometers (or 254 nanometers fixed mercury source), a column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles (U.S.P. designation L–1) 5 micrograms to 10 micrograms in diameter or equivalent, a flow rate of 1.5 milliliters per minute, and a known injection volume of 20 microliters. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.05M potassium phosphate buffer, pH 3.0.* Prepare a solution containing 6.8 grams of potassium phosphate monobasic per liter of distilled water. Adjust the solution to pH 3.0 with 1*M* phosphoric acid.

(*b*) *Mobile phase.* 0.05*M* potassium phosphate buffer, pH 3.0: methanol (4:1).

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams of aztreonam working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*b*) *Sample solution.* Transfer approximately 25 milligrams of the sample, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*c*) *Resolution test solution.* Dissolve 10 milligrams of [2*S*-[2*alpha*,3*beta*(*E*]]-2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene] amino]oxy]-2-methylpropanoic acid (E isomer) in 10 milliliters of working standard solution and dilute to 50-milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between the peak for aztreonam and the E isomer is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation ($S_R$in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculation.* Calculate the micrograms of aztreonam per milligram as follows:

$$\text{Micrograms of aztreonam per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the aztreonam peak in the chromatogram of the aztreonam working standard;

P_s=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

C_u=Milligrams of sample per milliliter of sample solution.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid I in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam and 39 milligrams of pyrogen-free L-arginine base per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5

percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[52 FR 4614, Feb. 13, 1987, as amended at 55 FR 11584, Mar. 29, 1990]

§ 455.10 Chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D-(—)-*threo*—1-*p*—nitrophenyl-2 -dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(iv) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(v) Its melting range is 151°±2° C.

(vi) Its absorptivity at 278 nanometers is 100 ±3 percent of that of the chloramphenicol working standard similarly treated.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 95 percent ethyl alcohol to obtain a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient distilled water to obtain a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) [Reserved]

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15

milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(6) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section, except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§ 455.10a Sterile chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D - (—) - *threo* - 1 - *p*—nitrophenyl - 2 - dichloracetamido - 1,3 - propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv)—(v) [Reserved]

(vi) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(vii) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(viii) Its melting range is 151°±2° C.

(ix) Its absorptivity at 278 nanometers is 100 ±3 percent of that of the chloramphenicol working standard similarly treated.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 50 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 95 percent ethyl alcohol to obtain a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient distilled water to obtain a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standards in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water

as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter. Apply sufficient heat to dissolve the chloramphenicol.

(4)—(5) [Reserved]

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(7) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0 decimeter polarimeter tube.

(8) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(9) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 45 FR 64568, Sept. 30, 1980; 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§ 455.11 Chloramphenicol palmitate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate is the white to grayish-white, tasteless palmitic acid ester of chloramphenicol. It is so urified and dried that:

(i) It contains not less than 555 micrograms nor more than 595 micrograms of chloramphenicol per milligram.

(ii) [Reserved]

(iii) Its melting range is 91°±4° C.

(iv) Its specific rotation in absolute ethyl alcohol at 25° C. is +23°±2°.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for chloramphenicol content, melting range, specific rotation, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Chloramphenicol content.* Proceed as directed in § 436.335 of this chapter.

(2) [Reserved]

(3) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of sample in a 25-milliliter, glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary to effect solution. Bring the solution to 25° C. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 49 FR 6093, Feb. 17, 1984; 50 FR 19921, May 13, 1985]

§ 455.12a Sterile chloramphenicol sodium succinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol sodium succinate is the light-yellow, water-soluble, ethanol-soluble sodium salt of the 3-monosuccinate ester of chloramphenicol. It is so purified and dried that:

(i) Its potency is not less than 650 and not more than 765 micrograms per milligram. If it is packaged for dispensing, its potency when reconstituted as directed in the labeling is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of chloramphenicol per milliliter that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv)—(v) [Reserved]

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 250 milligrams of chloramphenicol per milliliter is not less than 6.4 and not more than 7.0.

(viii) Its specific rotation in an aqueous solution containing 50 milligrams per milliliter at 25° C. is +6.5°±1.5°.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and specific rotation.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 8 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Working standard.* Dissolve an accurately weighed portion of the chloramphenicol working standard in sufficient distilled water to give a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wavelength of 278 nanometers.

(ii) *Procedure.* Dissolve an accurately weighed portion of the sample to be tested in sufficient distilled water to give a solution containing 30 micrograms of the sample per milliliter (estimated); and also if it is packaged for dispensing, reconstitute as directed in the labeling. Remove an accurately measured representative portion from each container and further dilute this portion with sufficient distilled water to give a concentration of 20 micrograms of chloramphenicol per milliliter (estimated). Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wave length of 276 nanometers. Calculate the micrograms per milligram of the dry powder as follows:

$$\text{Micrograms of chloramphenicol per milligram} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{potency of chloramphenicol working standard in the micrograms per milligram}}{\text{Absorbance of standard at 278 nanometers} \times \text{micrograms of sample per milliliter}}$$

Calculate the milligrams per milliliter of the reconstituted solution in the dispensing container as follows:

$$\text{Milligrams per milliliter of the reconstituted vial} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{labeled content of reconstituted vial in milligrams per milliliter}}{\text{Absorbance of standard at 278 nanometers} \times 20}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter.

(4)–(5) [Reserved]

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 250 milligrams of chloramphenicol per milliliter.

(8) *Specific rotation.* Dilute the sample with sufficient distilled water to give a solution containing approximately 50 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

[39 FR 19166, May 30, 1974, as amended at 39 FR 37486, Oct. 22, 1974; 45 FR 64568, Sept. 30, 1980; 50 FR 1504, Jan. 11, 1985; 50 FR 19921, May 13, 1985]

§ 455.15 Clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.5 percent.

(iii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(iv) It gives a positive identity test.

(v) Its content of the potassium salt of [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content.* Proceed as directed in § 436.351 of this chapter, using ambient temperature, an ultraviolet

detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.05M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4±0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix methanol: 0.05*M* sodium phosphate buffer, pH 4.4 (5:95 v/v) and stir or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Preparation of clavulanic acid working standard and sample solutions.* Accurately weigh and transfer into volumetric flasks sufficient clavulanic acid working standard or clavulanate potassium sample to obtain a final concentration of 250 micrograms per milliliter. To the clavulanic acid working standard, add sufficient amoxicillin trihydrate to provide a final concentration of 500 micrograms per milliliter. (The amoxicillin serves as an internal marker for system suitability testing.) Dissolve in water by shaking or ultrasonicating until solution becomes clear. Dilute the solutions as required to final volume with water. Use within 8 hours.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 550 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of clavulanic acid per milligram of sample as follows:

$$\text{Micrograms of clavulanic acid per milligram} = \frac{A_u \times P_s \times W_s \times 100}{A_s \times W_u \times (100 - m)}$$

where:

A_u=The clavulanic acid peak response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=The clavulanic acid peak response in the chromatogram of the clavulanic acid working standard);

P_s=Potency of the clavulanic acid working standard in micrograms per milligram;

W_u=Milligrams of sample;

W_s=Milligrams of standard; and

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(5) *Clavam-2-carboxylate content.* Proceed as directed in § 436.352 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials such as octadecyl silane bonded silica. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase. 0.1M Sodium phosphate buffer solution, pH 4.0.* Prepare a 0.1*M* aqueous solution of monobasic sodium phosphate and adjust to pH 4.0 with phosphoric acid.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Accurately weigh and

transfer into a 50-milliliter volumetric flask approximately 16 milligrams of clavam-2-carboxylate authentic sample. Dilute to volume and transfer 10 milliliters into a 100-milliliter flask. Dilute to volume with water.

(*b*) *Preparation of sample solution.* Accurately weigh 100 milligrams of the sample into a 10-milliliter flask. Dilute to volume with water.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) for the clavulanate standard peak is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 4,000 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and clavam-2-carboxylic acid peaks is satisfactory if it is greater than 1.0.

(*d*) *Coefficient of variation (Relative standard deviation).* The coefficient of variation ($S_R$in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.352(b) of this chapter.

(iv) *Calculations.* Calculate the percent of clavam-2-carboxylate content as follows:

$$\text{Percent clavam-2-carboxylate content} = \frac{\text{Mean sample height (or area)} \times \text{weight of standard} \times \text{P}}{\text{Mean peak height (or area) of standard} \times \text{weight of sample} \times 50}$$

where:

P=Percent clavam-2-carboxylic acid in the standard.

[49 FR 39674, Oct. 10, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§ 455.15a Sterile clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) Its pH of an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(vi) It gives a positive identity test.

(vii) Its [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) content is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content.* Proceed as directed in § 455.15(b)(1) of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams per milliliter of clavulanate potassium.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(7) *Clavam-2-carboxylate content.* Proceed as directed in § 455.15(b)(5) of this chapter.

[50 FR 33519, Aug. 20, 1985, as amended at 54 FR 11584, Mar. 29, 1990]

§ 455.20 Cycloserine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish compound. It has the

chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its residue on ignition is not more than 0.5 percent.

(vi) It gives a positive identity for cycloserine.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Colorimetric assay*—(*a*) *Stockstandard solution*. Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to give a solution containing 1,000 micrograms per milliliter. This solution may be used for 1 month if kept under refrigeration.

(*b*) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(*c*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(*d*) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100 milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to respective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 nanometers in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(*e*) Calculations:

Micrograms cycloserine per milligram = (Concentration in micrograms from calibration curve × 1,000)/Weight of original sample in milligrams.

(ii) *Microbiological turbidimetric assay*. Proceed as described in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution with a concentration 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in paragraph (b)(1)(i) of this section.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.40 Mupirocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mupirocin is nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-l-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-,[2*S*-[2α(*E*),3*B*,4*B*,5α[2*R**,3*R**(1*R**,2*R**)]]]-. It is a white to off-white crystalline solid. It is so purified and dried that:

(i) Its potency is not less than 920 micrograms per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.0 percent.

(iii) The pH of a saturated aqueous solution of mupirocin is not less than 3.5 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for mupirocin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 229 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as an octadecylsilane, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Use the resolution test solution to determine resolution in lieu of the working standard solution. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Acetonitrile.* Distilled in glass. Ultraviolet grade.

(B) *Phosphate buffer, pH 6.3.* Prepare a 0.05*M* sodium monobasic phosphate solution and adjust to pH 6.3 with 1.0*N* sodium hydroxide.

(C) *Mobile phase.* To 750 milliliters of 0.05*M*, pH 6.3 phosphate buffer, add 250 milliliters of acetonitrile. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution, test solutions*—(A) *Working standard solution.* Accurately weigh approximately 11 milligrams of the mupirocin working standard into a 100-milliliter volumetric flask. Dissolve the standard in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phosphate buffer. Mix well.

(B) *Sample solution.* Transfer approximately 11 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve the sample in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phospate buffer. Mix well.

(C) *Resolution test solution.* Acidify approximately 10 milliliters of the working standard solution with 6*N* hydrochloric acid to pH 2.0. Allow to stand at room temperature for about 2 hours. Neutralize this solution. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2_a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not more than 1.5.

(B) *Efficiency of the column.* From the number of theoretical plates (n) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;

n=Number of theoretical plates; and

d_P=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 20.0, equivalent to 1,500 theoretical plates for a 30-centimeter column of 10 micrometer particles.

(C) *Resolution factor.* The resolution factor (R_s) between the peak for mupirocin and its nearest eluting peak produced from its acid degradation is satisfactory if it is not less than 2.0. The chromatogram of the resolution test solution should show a significantly reduced mupirocin peak immediately preceded by a peak due to mupirocin degradation products. This degradation peak may appear as a single peak or be partially resolved showing a shoulder or two overlapping peaks.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of mupirocin per milligram of sample as follows:

$$\text{Micrograms of mupirocin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100-m)}$$

where:

A_u=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

P_s=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

C_u=Milligrams of mupirocin sample per milliliter of sample solution;

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter using a saturated aqueous solution.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in § 436.211(b)(2).

[55 FR 2641, Jan. 26, 1990; 55 FR 11110, Mar. 26, 1990; 55 FR 14378, Apr. 17, 1990]

§ 455.50 Calcium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 840 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vi) It demonstrates a positive color identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 5 milliliters of absolute ethyl alcohol and then dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of 1,000 micrograms (estimated) per milliliter. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a saturated aqueous suspension prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in §455.51a(b)(8).

(6) *Identity.* Proceed as directed in §455.51(b)(7).

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 43 FR 9801, Mar. 10, 1978; 50 FR 19921, May 13, 1985]

§455.51 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 6.0 percent.

(iv) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent, calculated on an anhydrous basis.

(vi) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vii) It demonstrates a positive color identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification: samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, specific rotation, identity and crystallinity.

(ii) Samples required on the batch; 10 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(5) *Residue on ignition.* Proceed as directed in §436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(6) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl

alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Identity*. (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(8) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.51a Sterile sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sodium novobiocin is the crystalline monosodium salt at a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 6.0 percent.

(vi) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent calculated on an anhydrous basis.

(viii) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(ix) It demonstrates a positive color identity test.

(x) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 600 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) [Reserved]

(5) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(8) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should develop in the benzene layer.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.70 Rifampin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin is a red-brown powder. It is 3-(4-methylpiperazinyliminomethyl) rifamycin SV. It is very slightly soluble in water, soluble in ethyl acetate and in methyl alcohol, and freely soluble in chloroform. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH is not less than 4.0 and not more than 6.0 in a 1 percent aqueous suspension.

(v) When calculated on the anhydrous basis, its absorptivity at 475 nanometers is 100±4 percent of that of the rifampin working standard, similarly treated.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution containing 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter, except dry the sample for 4 hours.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(5) *Absorptivity.* Determine the absorbance of the sample and standard

solutions in the following manner: Dissolve approximately 100 milligrams each of the sample and standard in a 100-milliliter volumetric flask containing 50 milliliters of absolute methyl alcohol, and dilute to volume with absolute methyl alcohol. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0, as listed in § 436.101(a)(1) of this chapter. Using a suitable spectrophotometer equipped with a 1-centimeter cell, immediately determine the absorption of each solution at 475 nanometers with the blank containing the same proportion of solution 1 and methyl alcohol as the sample and standard solutions. Calculate the absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\dfrac{\text{Absorbance of sample} \times \text{milligrams standard} \times (100 - m_1)}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100 - m_2)}}{} \times 100$$

where:

m_1=percent moisture in standard;
m_2=percent moisture in sample.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section, except use a 4 percent solution of the sample in chloroform and 0.1-millimeter matched absorption cells.

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.80a Sterile spectinomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile spectinomycin hydrochloride is the pentahydrated dihydrochloride salt of decahydro-4a, 7, 9-trihydroxy-2-methyl-6,8-bis(methylamino)4H-pyrano[2,3-b][1,4]benzodioxin-4-one. It is so purified and dried that:

(i) Its spectinomycin content is not less than 603 micrograms per milligram. If it is packaged for dispensing, its spectinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of spectinomycin that it is represented to contain.

(ii) Its microbiological activity is not less than 603 micrograms of spectinomycin per milligram.

(iii) It is sterile.

(iv) It is nonpyrogenic.

(v) [Reserved]

(vi) It contains no depressor substances.

(vii) Its moisture content is not less than 16 percent nor more than 20 percent.

(viii) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 3.8 nor more than 5.6. If it is packaged for dispensing, when reconstituted as directed in the labeling, its pH is not less than 4.0 nor more than 7.0.

(ix) It passes the identity test.

(x) Its residue on ignition is less than 1 percent.

(xi) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for spectinomycin content, microbiological activity, sterility, pyrogens, depressor substances, moisture, pH, identity, residue on ignition, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: eight packages, each containing approximately 300 milligrams and two containing not less than 3 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Spectinomycin content* (*vapor phase chromatography*). Proceed as directed in § 436.307 of this chapter; and also, if the batch is packaged for dispensing prepare the sample for assay as follows: Reconstitute the vial as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of spectinomycin content in a given volume of the resultant preparation remove an accurately measured representative portion from the container. Dilute the sample with water to a concentration equivalent to about 20 milligrams per milliliter of spectinomycin. Transfer 1.0 milliliter of the diluted sample to a 25-milliliter glass-stoppered Erlenmeyer flask and dry by lyophilization. Proceed as directed in § 436.307(d)(1)(ii) of this chapter. Calculate the spectinomycin content as follows:

$$\text{Milligrams of spectinomycin per dose} = \frac{R_u \times W_s \times D \times f}{R_s}$$

where:

$$R_u = \frac{\text{Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard)}}{\text{Area of internal standard peak}}$$

$$R_s = \frac{\text{Area of the spectinomycin standard peak}}{\text{Area of internal standard peak}}$$

W_s=Weight of the spectinomycin working standard in milligrams;

D=Dilution of the spectinomycin dose;

f=Potency of the spectinomycin working standard in milligrams of spectinomycin per milligram.

(2) *Microbiological activity* (*microbiological turbidimetric assay*). Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30.0 micrograms of spectinomycin per milliliter (estimated).

(3) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 50 milligrams of spectinomycin base per milliliter.

(5) [Reserved]

(6) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(7) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(8) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter, except, if it is packaged for dispensing, use the suspension obtained after reconstituting the drug as directed in the labeling.

(9) *Identity test*. Proceed as directed in § 436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

(10) *Residue on ignition*. Proceed as directed in § 436.207 of this chapter, using the method described in paragraph (b) of that section.

(11) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

§ 455.82a Sterile sulbactam sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile sulbactam sodium is sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4 dioxide. It is so purified and dried that:

(i) Its sulbactam potency is not less than 886 micrograms and not more than 941 micrograms per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1 percent.

(v) It is crystalline.

(vi) It passes the identity test for sulbactam sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 30 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 230 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0M Phosphoric acid.* Prepare by dissolving 67.5 milliliters of reagent grade phosphoric acid (85 percent) in distilled water and dilute to 1 liter.

(B) *0.005M Tetrabutylammonim hydroxide.* Dilute 6.6 milliliters of tetrabutylammonium hydroxide (40 percent) to 1,800 milliliters with distilled water. Adjust the pH to 5.0 with 1.0*M* phosphoric acid and dilute with distilled water to 2 liters.

(C) *Mobile phase.* Mix 350 milliliters of acetonitrile with 1,650 milliliters of 0.005*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chromatographic pumping system. (Slight adjustments in pH and/or acetonitrile content may be made to achieve the system suitability parameters defined in paragraph (b)(1)(iii) of this section.)

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of sulbactam working standard in sufficient mobile phase to give a stock solution of a known concentration containing about 1 milligram of sulbactam per milliliter.

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample in sufficient mobile phase to give a stock solution containing 1 milligram of sulbactam per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory for sulbactam if it is greater than 3,500 theoretical plates for a 30-centimeter column.

(C) *Resolution.* The resolution (*R*) between the peaks for sulbactam and penicillanic acid is satisfactory if it is not less than 3.8.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the micrograms of sulbactam per milligram of sample as follows:

$$\text{Micrograms of sulbactam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the sulbactam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the sulbactam peak in the chromatogram of the sulbactam working standard;

P_s=Sulbactam activity in the sulbactam working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of

sample solution; and
m=Percent moisture content of the sample.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in §436.32(b) of this chapter, using a solution containing 20 milligrams of sulbactam per milliliter.

(4) *Moisture*. Proceed as directed in §436.201 of this chapter.

(5) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

(6) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the sulbactam working standard.

[52 FR 42290, Nov. 4, 1987; 52 FR 45281, Nov. 25, 1987, as amended at 54 FR 47205, Nov. 13, 1989; 55 FR 11585, Mar. 29, 1990]

§455.85 Vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(v) It contains not more than 15 percent of factor A.

(vi) It gives a positive identity test for vancomycin.

(2) *Labeling*. It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, factor A content, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in §436.201 of this chapter.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(5) *Identity and factor A content*. Proceed as directed in §455.85a(b)(7).

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§455.85a Sterile vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in an aqueous solution containing 50 milligrams per milliliter

is not less than 2.5 and not more than 4.5.

(vii) Its heavy metals content is not more than 30 parts per million.

(viii) It contains not more than 15 percent of factor A.

(ix) It gives a positive identity test for vancomycin.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing, the vancomycin content of each immediate container is 500 milligrams of vancomycin.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, heavy metals, factor A content, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 12 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Identity and factor A content*—(i) *Preparation of the chromatogram*—(*a*) *Equipment.* (*1*) Chromatographic paper (Whatman No. 1 untreated filter paper).

(*2*) Equipment for descending paper chromatography (Mitchell tank).

(*b*) *Preparations of solutions*—(*1*) *Factor A.* Prepare a solution in distilled water to contain 1.33 milligrams of factor A per milliliter and further dilute with distilled water to prepare solutions containing 0.1 and 0.2 milligram of factor A per milliliter.

(*2*) *Vancomycin working standard solution.* Prepare a solution in distilled water to contain 1.33 milligrams of vancomycin per milliliter.

(*3*) *Known mixture of factor A and vancomycin.* Prepare a solution in distilled water to contain 0.2 milligram of factor A and 1.13 milligrams of vancomycin (estimated) per milliliter.

(*4*) *Sample.* Prepare two solutions of the sample in distilled water, each to contain 1.33 milligrams of vancomycin (estimated) per milliliter.

(*5*) *Solvent mixture.* Mix 300 milliliters of butyl alcohol, 150 milliliters of pyridine, and 200 milliliters of water in a large separatory funnel and shake well for 3 minutes. Let stand at room temperature. There should be no separation of layers.

(*c*) *Procedure.* Saturate the atmosphere in the tank with vapors of the

solvent mixture by placing 10 milliliters of the mixture in a trough in the bottom of the tank and closing tightly for 15 minutes. Prepare a sheet of chromatographic paper (8 inches x 8 inches) by carefully drawing a line of origin with a pencil 2 inches from one of the edges. Fold the paper along a straight line 1½ inches from the same edge of the paper. Starting 1 inch from the left-hand edge, establish points at 1-inch intervals along the line of origin on which to apply the solutions. Using a micropipette, apply the factor A solutions, the vancomycin solution, the known mixture solution, and the sample solutions by placing 5 microliters of each on separate spots. Properly identify the locations of the spots but avoid unnecessary handling of the paper. Allow the spots to dry spontaneously. Suspend the paper in the chamber so that the edge nearest the fold can be conveniently immersed in the solvent mixture contained in the top trough. Immerse the paper across its entire width to a depth sufficient to assure contact with the solvent mixture during the entire development time. Close the chamber tightly and allow the chromatograph to develop at room temperature for 6½ to 7 hours. Remove the paper and allow it to dry completely.

(ii) *Development by bioautograph*—(*a*) *Preparation of test organism* (*spore suspension*). The test organism is *Bacillus subtilis* (ATCC 6633),[1] test organism H, prepared as described in § 436.103 of this chapter, using the method described in paragraph (b)(2) of that section.

(*b*) *Preparation of plates*—(*1*) *Baselayer.* Add 42 milliliters of medium 2 described in § 436.102(b)(2) of this chapter to each Petri dish (25 millimeters x 150 millimeters) and allow to harden on a flat, level surface. To prevent condensation of excess moisture, raise the tops slightly while the agar hardens.

(*2*) *Seed layer.* Melt nutrient agar medium 2 described in § 436.102(b)(2) of this chapter. Accurately measure a sufficient quantity of the melted agar, cool to 48° C., and add the appropriate quantity of the spore suspension prepared as described in paragraph (b)(7)(ii)(*a*) of this section. Swirl the flask of inoculated agar to obtain a homogeneous suspension. Add 8 milliliters of this inoculated agar to each plate, spread evenly, and allow to harden on a flat, level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the entire surface of the plates.

(*c*) *Assay.* For each spot on the paper described in paragraph (b)(7)(i)(*c*) of this section, cut a strip 1.5 centimeters by approximately 14 centimeters with the center of each strip centered about the line of descent of the spot. Place all strips on plates with the aid of forceps within as short a period of time as possible. Use maximum spacing between strips. Insure complete contact so that the entire strip becomes uniformly moistened. Allow to stand for 30 minutes. Remove the strips and identify each strip location on the Petri dish. Incubate the plates for 16–18 hours at 37° C. Any zone of inhibition corresponding to factor A in the sample must not be greater than that of the 0.2 milligram-per-milliliter factor A standard. Also, the two areas of inhibition for the sample due to the presence of factor A and vancomycin must compare to the corresponding two areas of inhibition of the known mixture in their respective distances from their origins.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.86 Vancomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin is a tricyclic glycopeptide. It is a free flowing white to off-white colored powder. It is so purified and dried that:

(i) It contains not less than 925 micrograms of vancomycin per milligram, calculated on the anhydrous basis.

(ii) It contains not less than 92 percent vancomycin factor B and not more than 3 percent of any individual vancomycin related factor.

(iii) Its moisture content is not more than 20 percent.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(iv) Its heavy metals content is not more than 30 parts per million.

(v) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, chromatographic purity, moisture, heavy metals, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed sample of approximately 100 milligrams in a 100-milliliter volumetric flask and dissolve in approximately 50 milliliters of distilled water and 1.0 milliliter of 0.1*N* hydrochloric acid. Swirl or sonicate to dissolve the sample and bring to volume with distilled water. Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity.* Proceed as directed in § 436.366 of this chapter. The relative amount of vancomycin B is not less than 92 percent, and the relative amount of any related substance is not more than 3 percent.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc preparation as described in § 436.211(b)(1).

[59 FR 8400, Feb. 22, 1994]

§ 455.88 Rifabutin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifabutin is an amorphous red-violet powder. It is (9*S*, 12*E*, 14*S*, 15*R*, 16*S*, 17*R*, 18*R*, 19*R*, 20*S*, 21*S*, 22*E*, 24*Z*)-6,16,18, 20-tetrahydroxy-1′-isobutyl-14-methoxy-7, 9,15,17,19,21, 25-heptamethylspiro[9,4-(epoxypentadeca[1,11,13]trienimino)-2*H*-furo[2′, 3′:7,8] naphth[1,2-d]imidazole-2, 4′-piperidine]-5,10, 26-(3*H*, 9*H*)-trione-16-acetate. It is very slightly soluble in water, sparingly soluble in ethanol, and soluble in chloroform and methanol. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of rifabutin activity per milligram on an anhydrous basis.

(ii) Its content for the four major related substances detected by high-performance liquid chromatography (HPLC) is not more than 1.0 percent each. All other unknown related substances are not more than 0.5 percent. The total of all related substances is not more than 3.0 percent.

(iii) Its moisture content is not more than 2.5 percent.

(iv) Its *N*-isobutylpiperidone content is not more than 0.5 percent.

(v) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for rifabutin potency, related substances, moisture, *N*-isobutylpiperidone, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 ± 1 nanometers, an 11 centimeters X 4.7 millimeters (i.d.) column packed with microparticulate (5 to 7 micrometers in diameter) packing material such as octylsilane chemically bonded to porous silica (U.S. Pharmacopeia designation L7), a flow rate of about 1.0 milliliter per minute, and a manual or automatic injector capable of injecting 10 microliters. The retention time for rifabutin is between 9 and 11 minutes. Reagents; working standard, sample,

and resolution solutions; system suitability requirements; and calculations are as follows:

(i) *Reagents*—(A) *Hydrochloric acid, 2N*. Dilute 85 milliliters of hydrochloric acid (37 percent) with distilled water to 500 milliliters.

(B) *Potassium dihydrogen phosphate, 0.1M*. Prepare a solution containing 15.4 grams of potassium dihydrogen phosphate monohydrate (potassium phosphate monobasic) per liter of distilled water.

(C) *Sodium hydroxide, 2N*. Dissolve 8 grams of sodium hydroxide pellets in 100 milliliters of distilled water.

(D) *Mobile phase*. Acetonitrile:phosphate buffer, pH 6.5, 50:50. Mix equal quantities of acetonitrile and 0.1*M* potassium dihydrogen phosphate and adjust to an apparent pH of 6.5 ± 0.1 by dropwise addition of 2*N* sodium hydroxide. Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph. Slight adjustments of the mobile phase components ratio may be made in order to meet the system suitability requirements described in the system suitability tests in paragraph (b)(1)(iii) of this section.

(ii) *Preparation of working standard, sample, and resolution test solution*—(A) *Working standard solution*. Accurately weigh approximately 25 milligrams of the rifabutin working reference standard into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dissolve and dilute to volume with mobile phase and mix to obtain a solution having a known concentration of about 0.5 milligram of rifabutin per milliliter.

(B) *Sample solution*. Accurately weigh approximately 25 milligrams of sample into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dissolve and dilute to volume with mobile phase and mix to obtain a solution containing 0.5 milligram of rifabutin per milliliter (estimated).

(C) *Resolution test solution*. Dissolve approximately 10 milligrams of rifabutin in 2 milliliters of methanol and add 1 milliliter of 2*N* sodium hydroxide. Allow to stand for 3 to 4 minutes and then add 1 milliliter of 2*N* hydrochloric acid. Mix and dilute to 50 milliliters with mobile phase. Store aliquots of this solution in the frozen state for future use.

(iii) *System suitability requirements*. Using the apparatus and conditions described in this section, test the chromatographic system by injecting the resolution test solution. The chromatogram shows one major degradation peak and two minor degradation peaks eluting at relative retention times (RRT) of 0.5–0.6, 0.65–0.75, and 0.8–0.9, respectively, followed by the rifabutin peak.

(A) *Asymmetry factor*. The asymmetry factor (A_S) is satisfactory if it is not less than 1.0 and not more than 4.0 forther if a butin peak.

(B) *Efficiency of the column*. The absolute efficiency (h_r) is satisfactory if it is not more than 11 for the rifabutin peak, equivalent to 2,000 theoretical plates for a 11-centimeter column of 5-micrometer particles.

(C) *Resolution factor*. The resolution factor (R) between the peak for rifabutin and its closest eluting degradation product (generated in situ as described in paragraph (b)(1)(iii) of this section and eluting at RRT of 0.8–0.9) is satisfactory if it is not less than 1.3.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent of 5 replicate injections of the rifabutin working standard solution) is satisfactory if it is not more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of rifabutin per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of rifabutin per milligram} = \frac{A_U \times P_s \times 100}{A_s \times C_U \times (100 - m)}$$

where:

A_U = Area of the rifabutin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the rifabutin peak in the chromatogram of the rifabutin working standard;

P_S = Rifabutin activity in the rifabutin working standard solution in micrograms per milliliter;

C_U = Milligrams of sample per milliliter of

sample solution; and
m = Percent moisture content of the sample.

(2) *Related substances.* Proceed as directed in paragraph (b)(1) of this section for potency using the sample prepared as described in paragraph (b)(1)(ii)(B) of this section and calculating the amounts of related substances as follows.

(i) *Calculations.* Calculate the percentage of related substances as follows:

$$\text{Percent individual HPLC-related substance} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC-related substances} = \frac{A \times 100}{A_t}$$

where:
A_i = Area of the individual related substance peak;
A = The sum of areas of all peaks minus the area due to the rifabutin peak and solvent front peak; and
A_t = The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(ii) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *N-Isobutylpiperidone.* Proceed as directed in § 436.369 of this chapter.

(5) *Identity.* (i) Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(1) of that section using a 1 to 2 percent mixture in potassium bromide.

(ii) The identity of rifabutin is confirmed by the qualitative comparison of the HPLC of the sample to the rifabutin working standard as directed in paragraph (b)(1) of this section.

[59 FR 40807, Aug. 10, 1994; 59 FR 46479, Sept. 8, 1994]

§ 455.90a Sterile vidarabine monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vidarabine monohydrate is the monohydrate form of 9-β - D - arabinofuranosyl - 9H - purin - 6-amine. It is a white to off-white powder. It is so purified and dried that:

(i) Its vidarabine content is not less than 845 micrograms and not more than 985 micrograms of vidarabine per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) Its loss on drying is not less than 5 percent and not more than 7 percent.

(v) Its specific rotation in dimethylformamide at 25° C is —60.5°±4.5°.

(vi) It passes the identity test for vidarabine.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, this drug shall be labeled ''vidarabine''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for vidarabine content, sterility, loss on drying, specific rotation, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 200 milligrams.

(b) *Tests and methods of assay*—(1) *Vidarabine content.* Proceed as directed in § 436.325 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams.

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(5) *Specific rotation.* Using a solution containing 10 milligrams of vidarabine per milliliter in dimethylformamide and a polarimeter tube 1.0 decimeter in length, proceed as directed in § 436.210 of this chapter, except determine the specific rotation at 365 nanometers.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[42 FR 44224, Sept. 2, 1977; 43 FR 9802, Mar. 10, 1978, as amended at 44 FR 30334, May 25, 1979; 50 FR 19921, May 13, 1985]

Subpart B—Oral Dosage Forms

§ 455.110 Chloramphenicol capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol capsules are composed of chloramphenicol with or without one or more suitable and harmless diluents and lubricants. Each capsule contains 50, 100, or 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay; potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(1) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 100 milliliters of 95 percent ethyl alcohol. Blend for 2 minutes. Then add 400 milliliters of distilled water and blend again for 2 minutes. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Spectrophotometric assay*—(i) *Preparation of working standard solution.* Dissolve approximately 50 milligrams of the working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water.

(ii) *Procedure.* Place the contents of 10 capsules into a 250-milliliter volumetric flask. Add 50 milliliters of pure methyl alcohol to the flask and shake for at least 1 minute. Fill to volume with distilled water and mix thoroughly. Withdraw an aliquot and dilute with sufficient distilled water to give a concentration of 20 micrograms per milliliter. Using a suitable spectrophotometer equipped with a 1.0-centimeter cell and distilled water as the blank, determine the absorbance of the working standard and sample solutions at 278 nanometers. Calculate the potency as follows:

$$\text{Milligrams per capsule} = \frac{\text{Absorbance of sample} \times \text{labeled potency per capsule in milligrams}}{\text{Absorbance of standard}}$$

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§ 455.111 Chloramphenicol palmitate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate oral suspension is chloramphenicol palmitate and one or more suitable and harmless buffer substances, suspending agents, preservatives, colorings, and flavorings suspended in a suitable and harmless vehicle. Each milliliter contains chloramphenicol palmitate equivalent to 30.0 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. Its pH is not less than 4.5 nor more than 7.0. Its content of polymorph A crystals does not exceed 10 percent. The chloramphenicol palmitate used conforms to the standards prescribed by § 455.11(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol palmitate used in making the batch for chloramphenicol content, melting range, specific rotation, and crystallinity.

(*b*) The batch for chloramphenicol content, pH, and content of polymorph A crystals.

(ii) Samples required:

(*a*) The chloramphenicol palmitate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) Chloramphenicol content (high-pressure liquid chromatography). Proceed as directed in § 436.335 of this chapter, except prepare the sample solution and calculate the chloramphenicol content as follows:

(i) *Preparation of sample solution.* Transfer a portion of the sample equivalent to 150 milligrams of chloramphenicol into a 200-milliliter volumetric flask. Add 100 milliliters of methanol and 4 milliliters of glacial acetic acid. Shake and dilute to volume with methanol. Filter the solution through a glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter.

(ii) *Calculations.* Calculate the chloramphenicol content as follows:

$$\begin{matrix}\text{Milligrams of}\\ \text{chloramphenicol}\\ \text{per milliliter}\end{matrix} = \frac{(A)(W_s)(f)(4)}{(B)(1{,}000)(V)}$$

where:

A=Area of the chloramphenicol palmitate sample peak (at a retention time equal to that observed for the standard);

B=Area of the working standard peak;

W_s=Weight of standard in milligrams;

f=Micrograms of chloramphenicol activity per milligram of chloramphenicol palmitate working standard; and

V=Volume of sample in milliliters.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(3) *Content of polymorph A crystal.*—(i) *Preparation of standards*—(*a*) *Standard containing 20 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 4 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(*b*) *Standard containing 10 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 9 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(ii) *Preparation of sample.* Place 20 milliliters of thoroughly mixed oral suspension into a 50-milliliter centrifuge tube. Add 20 milliliters of water and mix. Centrifuge for 10 to 15 minutes at a speed not less than 18,000 revolutions per minute. Decant the supernatant liquid. Wash the residue as follows: Add 2 milliliters of water to the residue, mix to make paste, add 18 milliliters of water, and mix thoroughly. Centrifuge, decant the supernatant liquid, and wash the residue two more times. Remove the washed residue from the centrifuge tube and dry it at least 14 hours in a vacuum desiccator at room temperature.

(iii) *Procedure.* Weigh 150 to 200 milligrams of liquid petrolatum into an agate mortar and add about 100 milligrams of standard or sample. Mix with a small spatula and then mull thoroughly with a pestle until a uniform consistency is obtained. Adjust a suitable infrared spectrophotometer so that 100 percent transmittance is recorded over the range of 11.0 to 13.0 microns. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal cell holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles and when placed in the instrument it should give a percent transmittance of 20 to 30 percent at 12.3 microns. Place the cell in the infrared spectrophotometer and record the absorption spectrum from 11.0 to 13.0 microns.

(iv) *Treatment of spectra*—(*a*) *Standard containing 20 percent of polymorph A.*

Determine by inspection of the recorded spectrum the exact wavelengths of minimum absorption at approximately 11.3 and 12.65 microns. Also determine by inspection the exact wavelengths of maximum absorption at approximately 11.65 and 11.86 microns. In the following subdivision, references to these four nominal wavelengths are to the exact wavelengths observed on the particular instrument being used.

(*b*) *Standard containing 10 percent of polymorph A.* Draw a straight baseline between the minima occurring at 11.3 and 12.65 microns. Draw straight lines at 11.65 and 11.86 microns intersecting both the recorded spectrum and the baseline. Obtain the corrected absorbances at 11.65 and 11.86 microns and calculate the absorbance ratios as follows:

$$\text{Absorbance ratio} = \frac{{}^{S}11.65 - {}^{B}11.65}{{}^{S}11.86 - {}^{B}11.86}$$

where:

^{S}11.65=Absorbance value of recorded spectrum at 11.65 microns;

^{B}11.65=Absorbance value at point of intersection of the 11.65-micron line with the baseline;

^{S}11.86=Absorbance value of recorded spectrum at 11.86 microns;

^{B}11.86=Absorbance value at point of intersection of the 11.86-micron line with the baseline.

(*c*) *Sample.* Proceed as described in paragraph (b)(3)(iv)(*b*) of this section.

(v) *Calculation.* The absorbance ratio of the sample must be greater than the absorbance ratio of the standard containing 10 percent of polymorph A.

[39 FR 19166, May 30, 1974, as amended at 49 FR 6093, Feb. 17, 1984; 50 FR 19921, May 13, 1985]

§ 455.120 Cycloserine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, quality, and purity.* Cycloserine capsules are capsules composed of crystalline cycloserine, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains 250 milligrams of cycloserine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain. The loss on drying is not more than 1.0 percent. The cycloserine used conforms to the standards prescribed by § 455.20(a)(1).

(2) *Labeling.* In addition to the labeling prescribed by § 432.5 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, paraaminosalicylic acid, viomycin, pyrazinamide, or combinations of these drugs, and that the drug may cause serious reactions such as convulsive seizures and mental disturbances.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency, loss on drying, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for cycloserine content and loss on drying.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical colorimetric assay*—(*a*) *Reagents.* (*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of the 4.0 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily and store in amber bottle.

(*5*) Cycloserine standard solution—dilute an appropriate-sized aliquot of the

stock standard solution, prepared as directed in § 455.20(b)(1)(i)(*a*), in 0.1*N* sodium hydroxide to obtain a working standard solution containing 100 micrograms of cycloserine per milliliter.

(*b*) *Procedure.* Transfer the contents of 10 capsules into a 1,000-milliliter volumetric flask. Add 0.1*N* sodium hydroxide to dissolve the sample, and add sufficient 0.1*N* sodium hydroxide to measure 1,000 milliliters. Mix well and filter. Dilute an alliquot of the filtrate with sufficient 0.1*N* sodium hydroxide to give a concentration of 0.1 milligram per milliliter (estimated) and mix well. Pipette exactly 1.0 milliliter of the working standard solution and 1.0 milliliter of the sample solution into separate test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid and exactly 1.0 milliliter of oxidized nitroprusside reagent to each of the test tubes; then mix thoroughly. Allow the tubes to stand at room temperature for 10 to 15 minutes, in order that maximum color intensity may develop. Using a reagent blank, determine the absorbance of the solutions at 625 nanometers in a suitable spectrophotometer.

Calculation:

$$\text{Milligrams of cycloserine per capsule} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Labeled potency per capsule in milligrams}$$

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with sufficient sterile distilled water to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.150 Calcium novobiocin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin oral suspension is a suspension containing calcium novobiocin and one or more suitable and harmless diluents, preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 25 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The pH is not less than 6.0 and not more than 7.5. The calcium novobiocin used conforms to the standards prescribed by § 455.50(a)(1) (i), (iv), (v), (vi), and (vii). If sodium novobiocin is reacted with a suitable calcium salt to form calcium novobiocin, the sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1) (i), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, pH, crystallinity, identity, and specific rotation. If sodium novobiocin is used in making the batch: Potency, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium novobiocin or the sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Remove a representative sample of the sirup with a suitable

syringe and place into a high-speed glass blender with sufficient absolute ethyl alcohol to give a concentration (estimated) of 1,000 micrograms per milliliter. Blend for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 1.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.151 Sodium novobiocin oral dosage forms.

§ 455.151a Sodium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin tablets are tablets that contain sodium novobiocin, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each tablet contains 125 milligrams or 250 milligrams of novobiocin. The 125-milligram tablet contains 375 milligrams of sulfamethizole. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. Its loss on drying is not more than 3 percent. The tablets disintegrate within 1 hour. The sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, disintegration time.

(ii) Samples required:

(*a*) Sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter, using the method described in paragraph (e)(1) of that section.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.151b Sodium novobiocin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin capsules are gelatin capsules containing sodium novobiocin with a suitable and harmless filler and with or without a binder and a lubricant. Each capsule contains 100 milligrams or 250 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The loss on drying is not more than 6.0 percent. The sodium novobiocin used conforms to the standards prescribed by § 455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The sodium novobiocin used in making the capsules: 10 packages, each

containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.170 Rifampin oral dosage forms.

§ 455.170a Rifampin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin capsules are gelatin capsules containing rifampin with a suitable and harmless filler and with or without binders, lubricants, and stabilizers. Each sample contains 150 milligrams or 300 milligrams of rifampin. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of rifampin that it is represented to contain. Its loss on drying is not more than 3.0 percent. The rifampin used conforms to the standards prescribed by § 455.70(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The rifampin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol and blend for 3 minutes. Add 300 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19166, May 30, 1974. Redesignated at 40 FR 53997, Nov. 20, 1975, and amended at 46 FR 46314, Sept. 18, 1981; 50 FR 19921, May 13, 1985]

§ 455.170b Rifampin-isoniazid capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin-isoniazid capsules contain rifampin and isoniazid with a suitable and harmless filler and with or without binders, lubricants, and stabilizers in a gelatin capsule. Each capsule contains 300 milligrams of rifampin and 150 milligrams of isoniazid. Its rifampin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of rifampin that it is represented to contain. Its isoniazid content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of isoniazid that it is represented to contain. Its loss on drying is not more than 3.0 percent. The rifampin used conforms to the standards prescribed by § 455.70(a)(1). The isoniazid used conforms to the standards prescribed by the U.S.P.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The isoniazid used in making the batch for all U.S.P. specifications.

(*c*) The batch for rifampin content, isoniazid content, and loss on drying.

(ii) Samples required:

(*a*) The rifampin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Tests and methods of assay*—(1) *Rifampin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol and blend for 3 minutes. Add 300 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Isoniazid content*—(i) *Equipment*—(*a*) *Electronic voltmeter.* A vacuum tube voltmeter or pH meter capable of measuring potentials from 0 to 1,400 millivolts.

(*b*) *Platinum electrodes.* Use twin platinum electrodes.

(*c*) *Constant current potential source.* Polarize the platinum electrodes by means of a battery and a suitable resistance in series with the electrodes, or by a stable electronic power supply, so that the current flow is about 2.5 microamperes.

(*d*) *Titration vessel.* Use a 100-milliliter beaker.

(ii) *Reagents*—(*a*) Concentrated hydrochloric acid, reagent grade.

(*b*) 0.1*N* Bromine solution. Dissolve 3.0 grams of potassium bromate and 15.0 grams of potassium bromide in sufficient water to make 1 liter. Preserve in dark amber-colored, glass-stoppered bottles.

(*c*) 1.0*N* Potassium iodide. Dissolve 16.5 grams of potassium iodide in 100 milliliters of water.

(*d*) Starch iodide paste, T.S. (U.S.P.).

(*e*) 0.1*N* Sodium thiosulfate (U.S.P.).

(*f*) 0.1*N* Hydrochloric acid.

(*g*) Chloroform, reagent grade.

(iii) *Standardization of 0.1N bromine solution.* Measure accurately about 25 milliliters of the bromine solution into a 500-milliliter iodine flask and dilute with 120 milliliters of water. Add 5 milliliters of hydrochloric acid, insert the stopper in the flask, and shake it gently. Then add 5 milliliters of potassium iodide T.S., insert the stopper, shake the mixture, and allow it to stand for 5 minutes. Titrate the liberated iodine with standard 0.1*N* sodium thiosulfate U.S.P., adding starch iodide paste T.S./U.S.P. as the endpoint is approached. Calculate the normality of the bromine solution.

(iv) *Preparation of sample solution.* Empty the contents of not less than 10 capsules into a tared weighing bottle. Mix and weigh the powder. Calculate the average capsule weight content and accurately weigh a sample equivalent to approximately 100 milligrams of isoniazid. Transfer the sample to a 125-milliliter separatory funnel. Add 20 milliliters of 0.1*N* hydrochloric acid and shake well. Extract the acidic solution with six 25-milliliter portions of chloroform, combining any interfacial emulsion with the aqueous phase throughout the extraction procedure. Discard the chloroform extracts. Quantitatively transfer the acidic aqueous layer to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid.

(v) *Titration procedure.* Pipet 25 milliliters of the sample solution into the titration vessel and add 10 milliliters of concentrated hydrochloric acid. Adjust the volume to approximately 50 milliliters with water. Titrate potentiometrically at constant current with 0.1*N* bromine solution to a dead stop endpoint. Calculate the isoniazid content for the sample used and determine the isoniazid content for the average capsule weight as follows:

$$\text{Milligrams isoniazid per average capsule} = \frac{V \times N \times 34.29 \times 4 \times W}{S}$$

where:

V=Volume in milliliters of 0.1*N* bromine solution used to titrate the sample;

N=Normality of bromine solution;

W=Average capsule weight content in milligrams;

S=Weight of sample in milligrams.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[40 FR 53997, Nov. 20, 1975, as amended at 50 FR 19921, May 13, 1985]

§ 455.185 Vancomycin hydrochloride oral dosage forms.

§ 455.185a Vancomycin hydrochloride for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for oral solution is vancomycin hydrochloride packaged in a suitable dispensing container. It may contain a suitable stabilizing agent. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of grams of vancomycin that it is represented to contain. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 4.5. The vancomycin hydrochloride used conforms to the standards prescribed by § 455.85.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The vancomycin hydrochloride used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Empty the contents into an accurately measured volume of distilled water as directed in the labeling of the drug. Further dilute an aliquot with 0.1*M* passium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985. Redesignated at 51 FR 22072, June 18, 1986, and amended at 59 FR 8399, Feb. 22, 1994]

§ 455.185b Vancomycin hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride capsules contain vancomycin hydrochloride dispersed in polyethylene glycol. Each capsule contains either 125 milligrams or 250 milligrams of vancomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. Its moisture is not more than 8 percent. It passes the dissolution test. The vancomycin hydrochloride used conforms to the standards prescribed by § 455.85(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(*b*) The batch for potency, moisture, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The vancomycin hydrochloride used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed

glass blender jar with sufficient distilled water to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section, except:

(i) Remove gelatin coating before grinding the capsules; and

(ii) Use solvent C in lieu of solvent A.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity *Q* (the amount of vancomycin dissolved) is 85 percent within 45 minutes.

[51 FR 22072, June 18, 1986, as amended at 55 FR 11585, Mar. 29, 1990]

§ 455.188 Rifabutin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifabutin capsules are gelatin capsules containing rifabutin with a suitable and harmless filler and with or without binders, lubricants, and stabilizers. Each capsule contains rifabutin equivalent to 150 milligrams of rifabutin. Its rifabutin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of rifabutin that it is represented to contain. Its content of the four major related substances detected by high-performance liquid chromatography (HPLC) is not more than 1.0 percent each. All other unknown related substances are not more than 0.5 percent. The total of all related substances is not more than 4.5 percent. It passes the dissolution test if the quantity (Q) dissolved is 75 percent at 45 minutes. It passes the identity test. The rifabutin used conforms to the standards prescribed by § 455.88(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The rifabutin used in making the batch for potency, related substances, moisture, *N*-isobutylpiperidone, and identity.

(B) The batch for content, related substances, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The rifabutin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Rifabutin content.* Proceed as directed in § 455.88(b)(1), preparing the sample solution and calculating the rifabutin content as follows:

(i) *Preparation of sample solution.* Empty 20 capsules, collecting the contents quantitatively. Weigh the powder and determine the average capsule fill weight. Mix the powder and accurately weigh a portion containing the equivalent of about 25 milligrams of rifabutin into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dilute to volume with mobile phase and mix to yield a solution containing 0.5 milligram of rifabutin per milliliter (estimated). Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter prior to injection into the chromatographic system.

(ii) *Calculations.* Calculate the rifabutin content as follows:

$$\text{Milligrams of rifabutin per capsule} = \frac{A_U \times C_S \times P_S \times W_a}{A_S \times C_U \times 1{,}000}$$

where:

A_U = Area of the rifabutin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the rifabutin peak in the chromatogram of the rifabutin working standard;

C_S = Milligrams of rifabutin working standard per milliliter of standard solution;

C_U = Milligrams of sample per milliliter of sample solution;

P_S = Rifabutin activity in the rifabutin working standard solution in micrograms per milliliter; and

W_a = Average capsule fill weight in milligrams.

(2) *Related substances.* Proceed as directed in paragraph (b)(1) of this section for rifabutin content using the sample prepared as described in paragraph (b)(1)(i) of this section and calculating the amounts of related substances as follows.

(i) *Calculations.* Calculate the percentage of related substances as follows:

$$\text{Percent individual HPLC-related substance} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC-related substances} = \frac{A \times 100}{A_t}$$

where:

A_i = Area of the individual related substance peak;

A = The sum of areas of all peaks minus the area due to the rifabutin peak and solvent front peak; and

A_t = The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(ii) [Reserved]

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity (Q) (the amount of rifabutin activity dissolved) is 75 percent within 45 minutes.

(4) *Identity.* (i) The retention time of the rifabutin response in the HPLC procedure described in paragraph (b)(1) of this section as applied to the sample solution compares qualitatively to that of the rifabutin reference standard.

(ii) The identity of rifabutin capsules is also confirmed by the spectrophotometric identity test described in § 436.370 of this chapter.

[59 FR 40808, Aug. 10, 1994]

Subpart C—Injectable Dosage Forms

§ 455.204 Aztreonam injectable dosage forms.

§ 455.204a Aztreonam for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam for injection is a dry mixture of aztreonam and arginine. Its potency is satisfactory if each milligram of aztreonam for injection contains not less than 900 micrograms and not more than 1,050 micrograms of aztreonam when corrected for arginine content and moisture content. Its aztreonam immediate container fill (content) is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. Its pH in an aqueous solution containing 100 milligrams of aztreonam per milliliter is not less than 4.5 and not more than 7.5. The aztreonam used conforms to the standards prescribed by § 455.4a(a)(1), except if the aztreonam for injection is manufactured by lyophilization, in which case the aztreonam need not be sterile.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The aztreonam used in making the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity. If the aztreonam for injection is made by lyophilization, the aztreonam need not be tested for sterility.

(*b*) The batch for aztreonam potency, aztreonam content, sterility, pyrogens. moisture. and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The aztreonam used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency and content.* Determine both micrograms of aztreonam per milligram of sample and milligrams of aztreonam per container. Proceed as

directed in § 436.361 of this chapter, except in addition to the column described in paragraph (a)(4) of that section, use a 5- to 50-centimeter saturator column having an inside diameter of 2 to 4.6 millimeters and packed with approximately 37 micrometer silica; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 206 nanometers, and a column packed with Chromegabond Diol (dihydroxypropane chemically bonded to porous silica), 5 to 10 micrometers or equivalent. Mobile phase, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Acetonitrile:0.01*M* ammonium phosphate, pH 2.0. Transfer 1.15 grams of ammonium phosphate monobasic to a 1-liter volumetric flask. Add about 800 milliliters of distilled water and sonicate to aid dissolution. Adjust the solution to pH 2.0 with o-phosphoric acid, 85 percent. Dilute the solution to volume with distilled water and mix well. Transfer about 250 milliliters of this solution and 750 milliliters of acetonitrile to a suitably sized container and mix well.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams each of the aztreonam working standard and the arginine working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase (primary working standard solution). Further dilute with mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*b*) *Sample solutions.* Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(ii)(*b*)(*1*) and (*2*) of this section.

(*1*) *Potency (micrograms of aztreonam per milligram).* Accurately weigh the container contents by difference and quantitatively transfer it to a 100-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase. Further dilute in mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*2*) *Content (milligrams of aztreonam per container).* If packaged in containers with capacities of less than 100 milliliters, reconstitute the sample as directed in the labeling, using distilled water in lieu of reconstituting fluid. If packaged in bottles with capacities of 100 milliliters or greater, reconstitute with 10 milliliters of distilled water. Withdraw the total contents of each container or bottle and dilute with mobile phase to a concentration of 0.2 milligram of aztreonam per milliliter (estimated).

(*c*) *Resolution test solution.* Dissolve 10 milligrams of open ring aztreonam, [[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-(sulfoamino)-butanoic acid, in 10.0 milliliters of primary standard solution. Further dilute 5 milliliters of this solution to 25.0 milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) of the aztreonam peak is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between aztreonam peak and open ring aztreonam is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—(*a*) *Potency (micrograms per milligram).* (*1*) Calculate the micrograms of aztreonam per milligram (uncorrected) as follows:

$$\text{Micrograms of aztreonam per milligram (uncorrected)} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the aztreonam peak in the chromatogram of the working standard;
P_s=Aztreonam activity in the working standard solution in micrograms per milliliter; and
C_u=Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the micrograms of arginine per milligram as follows:

$$\text{Micrograms of arginine per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

A_u=Area of the arginine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the arginine peak in the chromatogram of the working standard;
P_s=Arginine activity in the working standard solution in micrograms per milliliter; and
C_u=Milligrams of sample per milliliter of sample solution.

(*3*) Calculate the micrograms of aztreonam per milligram (corrected) as follows:

$$\text{Micrograms of aztreonam per milligram (corrected)} = \frac{\text{Micrograms of aztreonam per milligram (uncorrected)} \times 1{,}000}{1{,}000 - [\text{Micrograms of arginine per milligram} + (\text{Percent moisture}) \times 10]}$$

(*b*) *Content (milligrams of aztreonam per container).* Calculate the aztreonam content of the container as follows:

$$\text{Milligrams of aztreonam per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the aztreonam peak in the chromatogram of the working standard;
P_s=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and
d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of aztreonam per milliliter.

[52 FR 4615, Feb. 13, 1987; 52 FR 8550, Mar. 18, 1987. Redesignated at 54 FR 40385, Oct. 2, 1989, and amended at 54 FR 41824, Oct. 12, 1989; 55 FR 11585, Mar. 29, 1990]

§ 455.204b Aztreonam injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam injection is a frozen aqueous iso-osmotic solution of aztreonam and arginine. Each milliliter contains aztreonam equivalent to either 10 milligrams, 20 milligrams, or 40 milligrams. Its aztreonam content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 7.5. It passes the identity test. The aztreonam used conforms to the standards prescribed by § 455.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The aztreonam used in making the batch for potency, moisture, residue on ignition, heavy metals, and identity.

(B) The batch for aztreonam potency, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The aztreonam used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Potency.* Proceed as directed in § 436.361 of this chapter, except in addition to the column described in paragraph (a)(4) of that section, use a 5- to 50-centimeter saturator column having an inside diameter of 2 to 4.6 millimeters and packed with approximately 37 micrometer silica, and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating a wavelength of 206 nanometers, and a column packed with Chromegabond Diol (dihydroxypropane chemically bonded to porous silica), 5 to 10 micrometers or equivalent. Mobile phase, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations as follows:

(i) *Mobile phase.* Acetonitrile: 0.01*M* pH 2.0 ammonium phosphate (75:25). Transfer 1.15 grams of ammonium phosphate monobasic to a 1-liter volumetric flask. Add about 800 milliliters of distilled water and sonicate to aid dissolution. Adjust the solution to pH 2.0 with *o*-phosphoric acid, 85 percent. Dilute the solution to volume with distilled water and mix well. Transfer about 250 milliliters of this solution and 750 milliliters of acetonitrile to a suitable-sized container and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Transfer approximately 25 milligrams each of the aztreonam working standard and the arginine working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase (primary working standard solution). Further dilute with mobile phase to obtain a solution containing 0.2 milligram of aztreonam per milliliter.

(B) *Sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient mobile phase to obtain a solution containing 0.2 milligram of aztreonam per milliliter (estimated).

(C) *Resolution test solution.* Dissolve 10 milligrams of open ring aztreonam, 2-[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-(sulfoamino)-butanoic acid, in 10.0 milliliters of primary standard solution. Further dilute 5 milliliters of this solution to 25.0 milliliters with mobile phase.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) of the aztreonam peak is satisfactory if it is not more than 2 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(C) *Resolution.* The resolution (*R*) between the aztreonam peak and open ring aztreonam is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.361(b) of this chapter. Alternative chromatographic conditions are acceptable, provided reproducibility and resolution are comparable to the system. However, the

sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations:* Calculate the milligrams of aztreonam per milliliter of sample as follows:

$$\text{Milligrams of aztreonam per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the aztreonam peak in the chromatogram of the working standard;

P_s=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of aztreonam per kilogram.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high-performance liquid chromatogram of the sample is determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the aztreonam working standard.

[54 FR 40385, Oct. 2, 1989]

§ 455.210 Chloramphenicol injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol injection is chloramphenicol, with or without one or more suitable and harmless buffer substances, dissolved in one or more suitable and harmless solvents. Each milliliter contains 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.7 and not more than 5.0. The chloramphenicol used conforms to the standards prescribed by § 455.10a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of eight immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add the contents of each container directly to the dry filter, thus eliminating the preliminary solubilization step.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams per milliliter.

(4)–(5) [Reserved]

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[39 FR 19166, May 30, 1974, as amended at 45 FR 64568, Sept. 30, 1980; 48 FR 3961, Jan. 28, 1983; 48 FR 7440, Feb. 22, 1983; 50 FR 19921, May 13, 1985]

§ 455.212 Sterile chloramphenicol sodium succinate.

The requirements for certification and the tests and methods of assay for sterile chloramphenicol sodium succinate packaged for dispensing are described in § 455.12a.

[43 FR 9801, Mar. 10, 1978]

§ 455.230 Moxalactam disodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Moxalactam disodium for injection is a dry mixture of moxalactam disodium and mannitol. Its moxalactam content is satisfactory if it is not less than 90 percent and not more tha 120 percent of the number of milligrams of moxalactam that it is represented to contain. The moxalactam content of the dry mixture is not less than 722 micrograms of moxalactam per milligram. The ratio of R-isomer to S-isomer is not less than 0.8 and not more than 1.4. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 4.5 and not more than 7.0. It passes the identity test for moxalactam.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for moxalactam content, isomer ratio, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required on the batch:

(*a*) For all tests except sterility: A minimum of 10 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Moxalactam content; isomer ratio.* Proceed as directed in § 436.332 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of moxalactam.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity.* Proceed as directed in § 436.333 of this chapter.

[46 FR 61070, Dec. 15, 1981, as amended at 50 FR 19921, May 13, 1985]

§ 455.251 Sodium novobiocin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin for injection is sodium novobiocin with or without one or more suitable solubilizing agents, preservatives, and diluents. Each vial contains 500 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. It is sterile and nonpyrogenic. Its loss on drying is not more than 6.0 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.5 and not more than 8.5. The sodium novobiocin used conforms to the standards prescribed by § 455.51a(a)(1) (i), (iii), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32 of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) [Reserved]

(5) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the sample after reconstituting as directed in the labeling.

[39 FR 19166, May 20, 1974, as amended at 46 FR 25608, May 8, 1981; 50 FR 19921, May 13, 1985]

§ 455.270 Rifampin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rifampin for injection is a dry mixture of rifampin, sodium formaldehyde sulfoxylate, and sodium hydroxide. Its potency is 600 milligrams per vial. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rifampin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 7.8 and not more than 8.8. It passes the identity test. The rifampin used conforms to the standards prescribed by § 455.70(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(B) The batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research.

(A) The rifampin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the preparation, withdraw an accurately measured volume from each container. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1) to give a stock solution of 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of rifampin per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using a concentration of 60 milligrams of rifampin per milliliter.

(6) *Identity.* Proceed as directed in §436.365 of this chapter.

[54 FR 38375, Sept. 18, 1989]

§455.280a Sterile spectinomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile spectinomycin hydrochloride packaged for dispensing are described in §455.80a.

§455.285 Vancomycin hydrochloride injectable dosage forms.

§455.285a Sterile vancomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile vancomycin hydrochloride packaged for dispensing are described in §455.85a.

§455.285b Vancomycin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for injection is a dry mixture of vancomycin hydrochloride and a suitable stabilizing agent. It contains not less than 925 micrograms of vancomycin per milligram, calculated on an anhydrous basis. Its vancomycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. It contains not less than 88 percent vancomycin factor B. It contains not more than 4 percent of any individual vancomycin related factor. It is sterile. It is nonpyrogenic. Its moisture content is not more than 5 percent. The pH of an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5. Its heavy metals content is not more than 30 parts per million. It gives a positive identity test. The vancomycin hydrochloride used conforms to the standards prescribed by §455.85(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Request for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(B) The batch for vancomycin potency, vancomycin content, chromatographic purity, sterility, pyrogens, moisture, pH, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The vancomycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Test and methods of assay*—(1) *Vancomycin potency and content.* Determine both micrograms of vancomycin per milligram of sample and milligrams of vancomycin per container. Proceed as directed in §435.105 of this chapter, preparing the sample solution as follows:

(i) *Preparation of sample solution.* Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(i) (A) and (B) of this section.

(A) *Micrograms of vancomycin per milligram.* Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient distilled water to obtain a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(B) *Milligrams of vancomycin per container.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or,

if the labeling specifies the amount of vancomycin content in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity*. Proceed as directed in § 436.366 of this chapter. The relative amount of vancomycin B is not less than 88 percent and the relative amount of any related substance is not more than 4 percent.

(3) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(4) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

(8) *Identity*. Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc preparation as described in paragraph (b)(1) of that section.

[54 FR 20384, May 11, 1989; 54 FR 22838, May 28, 1989]

§ 455.285c Vancomycin hydrochloride injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vancomycin hydrochloride injection is a frozen, aqueous, iso-osmotic solution of vancomycin hydrochloride and a tonicity adjusting agent. Each milliliter contains vancomycin hydrochloride equivalent to 5 milligrams of vancomycin. Its vancomycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. It contains not less than 88 percent vancomycin factor B. It contains not more than 4 percent of any individual vancomycin related factor. It is sterile. It contains not more than 0.33 U.S.P. Endotoxin Unit per milligram of vancomycin hydrochloride. Its pH is not less than 3.0 and not more than 5.0. The vancomycin used conforms to the standards prescribed by § 455.86.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, this drug shall be labeled ''vancomycin hydrochloride injection.''

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The vancomycin used in making the batch for vancomycin potency, chromatographic purity, moisture, heavy metals, and identity.

(B) The batch for vancomycin content, chromatographic purity, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The vancomycin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*. Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Vancomycin content*. Proceed as directed in § 436.105 of this chapter, preparing the sample solution as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity*. Proceed as directed in § 436.366 of this chapter. The relative amount of vancomycin B is not less than 88 percent and the relative amount of any related substance is not more than 4 percent.

(3) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1), except use sterile distilled water in lieu of diluting fluid A.

(4) *Bacterial endotoxins*. Proceed as directed in the U.S.P. bacterial endotoxins test. The specimen under test contains not more than 0.33 U.S.P. Endotoxin Unit per milligram of vancomycin hydrochloride.

(5) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(6) *Identity*. The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section compares qualitatively to that of the vancomycin working standard.

[59 FR 8400, Feb. 22, 1994]

§ 455.290 Vidarabine monohydrate for infusion.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vidarabine monohydrate for infusion contains in each milliliter vidarabine monohydrate equivalent to 187.4 milligrams of vidarabine in an aqueous suspension containing suitable and harmless buffers and preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of vidarabine that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no histamine or histamine-like substances. Its pH is not less than 5.0 and not more than 6.2. The vidarabine monohydrate used conforms to the standards prescribed by § 455.90a (a)(1).

(2) *Labeling*. In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''vidarabine for infusion''.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The vidarabine monohydrate used in making the batch for vidarabine content, loss on drying, specific rotation, and identity.

(*b*) The batch for vidarabine content, sterility, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) The vidarabine monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Vidarabine content*. Proceed as directed in § 436.325 of this chapter, except prepare the sample solution and calculate the vidarabine content as follows:

(i) *Preparation of sample solution*. Using a suitable hypodermic needle and syringe, transfer 2 milliliters of the well-shaken suspension to a 500-milliliter volumetric flask. Add approximately 50 milliliters of distilled water and 5 milliliters of glacial acetic acid. Warm on a steam bath for 15 minutes to dissolve the vidarabine. Cool to room temperature and dilute to volume with distilled water. Transfer 4 milliliters to a 25-milliliter volumetric flask and dilute to volume with distilled water.

(ii) *Calculations*. Calculate the vidarabine content as follows:

$$\begin{matrix}\text{Milligrams of}\\ \text{vidarabine}\\ \text{per milliliter}\end{matrix} = \frac{A \times W_s \times f \times 125}{B \times 1{,}000 \times 16,}$$

where:

A=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);

B=Area of the standard peak;

W_s=Weight of the standard in milligrams; and

f=Potency of standard in micrograms per milligram.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of vidarabine per milliliter.

(4) *Histamine*. Proceed as directed in § 436.35 of this chapter. Apply sufficient heat to dissolve the vidarabine.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

[44 FR 1374, Jan. 5, 1979, as amended at 44 FR 30334, May 25, 1979; 50 FR 19921, May 13, 1985]

Subpart D—Ophthalmic Dosage Forms

§ 455.310 Chloramphenicol ophthalmic dosage forms.

§ 455.310a Chloramphenicol ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ophthalmic solution contains in each milliliter 5 milligrams of chloramphenicol with or without one or more suitable and harmless preservatives, buffer substances, and surfactants, in an aqueous solution. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 3 nor more than 6; however, if the solution is buffered, its pH is not less than 7.0 nor more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[39 FR 19166, May 30,1974, as amended at 43 FR 59057, Dec. 19, 1978; 46 FR 46313, Sept. 18, 1981; 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§ 455.310b Chloramphenicol for ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol for ophthalmic solution contains 25 milligrams of chloramphenicol with one or more suitable and harmless buffer substances. When reconstituted as directed in the labeling, its potency is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 7.1 and not more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each

containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods:

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay*. Reconstitute the sample as directed in the labeling and dilute a 1.0-milliliter aliquot in sufficient distilled water to obtain a solution containing 20 micrograms of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to obtain a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers. Calculate the potency of the sample as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{\text{Absorbance of sample} \times \text{labeled potency per milliliter in milligrams}}{\text{Absorbance of standard}}$$

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

[49 FR 6093, Feb. 17, 1984, as amended at 50 FR 19921, May 13, 1985]

§ 455.310c Chloramphenicol ointment (chloramphenicol cream).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Chloramphenicol ointment is chloramphenicol in a suitable and harmless ointment base, with or without suitable and harmless buffer substances, dispersing and suspending agents. It may contain cortisone or a suitable derivative of cortisone. If such base is water-miscible, it shall contain a suitable and harmless preservative. Its potency is not less than 1.0 milligram per gram. If it is intended for ophthalmic use, it is sterile. The chloramphenicol used conforms to the requirements of § 455.10a(a)(1), except paragraphs (a)(1) (ii), (iii), and (v) of that section. The chloramphenicol used in making the chloramphenicol ophthalmic ointment conforms to the requirements of § 455.10a(a)(1), except paragraphs (a)(1) (iii) and (v) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging*. Unless it is packaged in a single dose container, chloramphenicol ointment shall be packaged in collapsible tubes, which shall be well-closed containers as defined by the U.S.P., and shall not be larger than the ⅛-ounce size if such ointment is represented for ophthalmic use, and in no case larger than the 2-ounce size, except that if it is labeled solely for hospital use it may be packaged in immediate containers of glass which meet the test for tight containers as defined by the U.S.P. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in

applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by § 201.100 of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container the statement ''Expiration date _____'', the blank being filled in with the date that is 60 months, or 24 months if it is packaged in an immediate container other than tin or glass, or 12 months if the ointment base is water miscible, after the month during which the batch was certified.

(ii) If it contains one of the active ingredients specified in paragraph (a)(1) of this section, after the name ''chloramphenicol ointment'', wherever it appears, the name of the active ingredient, in juxtaposition with such name.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting point, and absorptivity.

(*b*) The batch for potency and for sterility if the ointment is intended for ophthalmic use.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers if it is packaged in immediate containers of tin or glass; a minimum of 20 immediate containers if it is packaged in immediate containers other than tin or glass.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows:

(i) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient distilled water to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of petroleum ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the aqueous layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the aqueous extractives in a suitable volumetric flask and dilute to volume with distilled water. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated). The potency of chloramphenicol ointment is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(2), except use 100 milligrams in lieu of 300 milligrams of solids.

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 44 FR 10380, Feb. 20, 1979; 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

§455.310d Chloramphenicol-polymyxin ointment.

(a) *Requirements for certification.* Chloramphenicol-polymyxin ointment conforms to all requirements and is subject to all procedures prescribed by §455.310c(a) for chloramphenicol ointment, except that:

(1) It contains not less than 10,000 units of polymyxin B per gram. The polymyxin B used conforms to the requirements prescribed for polymyxin B by §444.170a(a)(1) of this chapter.

(2) In lieu of the labeling prescribed by §455.310c(a)(3)(i)(*a*), each package shall bear on the outside wrapper or container and the immediate container, the statement ''Expiration date _____'', the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by this section: *Provided however,* That such expiration date may be omitted from the immediate container if it contains a single dose and it is packaged in an individual wrapper or container.

(3) In addition to complying with the requirements of §455.310c(a)(4), a person who requests certification of a batch shall submit with his request a statement showing the batch mark and (unless previously submitted) the results and date of the latest tests and assays of the polymyxin used in making the batch for potency. He shall also submit in connection with his request a sample consisting of not less than 6 packages of ointment and (unless it was previously submitted) a sample consisting of 5 packages containing approximately equal portions of not less than 0.5 gram each of the polymyxin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chloramphenicol content.* Proceed as directed in §455.310c(b). Its chloramphenicol content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in §444.170a(b)(2)(i) of this chapter, except in lieu of the directions in §444.170a(b)(2)(i)(*g*) of this chapter for the preparation of the sample, prepare the sample as follows: Place an accurately weighed sample (usually approximately 1.0 gram) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether, and shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer, pH 6.0 and shake. Remove the buffer layer and repeat the extraction with three additional 25-milliliter portions of buffer. Combine the extractives and make the proper estimated dilutions, using the buffer solution, except that, if the sample contains a water-soluble base, place an accurately weighed representative sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a final volume of 200 milliliters. Using a highspeed blender, blend the mixture for 2 minutes to 3 minutes and then make the proper estimated dilutions with 10 percent phosphate buffer pH 6.0. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units per gram that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams of solids.

[39 FR 19166, May 30, 1974, as amended at 47 FR 23443, May 28, 1982; 50 FR 19921, May 13, 1985]

§455.310e Chloramphenicol - hydrocortisone acetate for ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Chloramphenicol-hydrocortisone acetate for ophthalmic suspension contains 12.5 milligrams of chloramphenicol and 25 milligrams of hydrocortisone acetate with one or more suitable and harmless buffer substances, preservatives, and diluents. When reconstituted as directed in the labeling, its potency is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 7.1 and not more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods:

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay*. Reconstitute the sample as directed in the labeling and dilute a 1.0-milliliter aliquot in sufficient distilled water to obtain a solution containing 20 micrograms of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to obtain a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers. Calculate the potency of the sample as follows:

Milligrams of chloramphenicol per milliliter=Absorbance of sample X labeled potency per milliliter in milligrams/Absorbance of standard.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

[49 FR 6093, Feb. 17, 1984]

§ 455.390 Vidarabine monohydrate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vidarabine monohydrate ophthalmic ointment contains in each gram vidarabine monohydrate equivalent to 28.11 milligrams of vidarabine in a suitable and harmless base. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of vidarabine that it is represented to contain. It is sterile. It passes the test for metal particles. The vidarabine monohydrate used conforms to the standards prescribed by § 455.90a(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''vidarabine ophthalmic ointment''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:k

(*a*) The vidarabine monohydrate used in making the batch for vidarabine content sterility, loss on drying, specific rotation, and identity.

(*b*) The batch for vidarabine content, sterility, and metal particles.

(ii) Samples required:

(*a*) The vidarabine monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Vidarabine content.* Proceed as directed in § 436.325 of this chapter, except prepare the sample solution and calculate the vidarabine content as follows:

(i) *Preparation of sample solution.* Accurately weigh a portion of the sample containing the equivalent of approximately 12 milligrams of vidarabine (estimated) into a 100-milliliter volumetric flask. Add approximately 80 milliliters of distilled water and heat for 15 minutes on a steam bath. Shake to dissolve the vidarabine and, while the solution is still hot, add 10 milliliters of heptane to dissolve the ointment base. Swirl gently until the ointment base is dissolved. Cool to room temperature and dilute the aqueous phase to volume with distilled water. Discard the heptane phase and mix the solution.

(ii) *Calculations.* Calculate the vidarabine content as follows:

$$\text{Percent vidarabine} = \frac{A \times W_s \times f}{(B \times W_u \times 10)}$$

where:

A=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);

B=Area of the standard peak;

W_s=Weight of standard in milligrams;

W_u=Weight of sample in milligrams; and

f=Potency of standard in micrograms per milligram.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[42 FR 44224, Sept. 2, 1977, as amended at 44 FR 30335, May 25, 1979; 50 FR 19921, May 13, 1985]

Subpart E—Otic Dosage Forms

§ 455.410 Chloramphenicol otic.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol otic is a solution of chloramphenicol in a suitable and harmless vehicle. Each milliliter contains 5.0 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its moisture content is not more than 2 percent. Its pH is not less than 4 and not more than 8. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain the following:

(i) Results of tests and asays on—

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity; and

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of

the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample diluted with an equal volume of distilled water.

[44 FR 5881, Jan. 30, 1979, as amended at 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

Subpart F—Dermatologic Dosage Forms

§ 455.510 Chloramphenicol dermatologic dosage forms.

§ 455.510a Chloramphenicol ointment (chloramphenicol cream).

The requirements for certification and the tests and methods of assay for chloramphenicol ointment (chloramphenicol cream) are described in § 455.310c.

§ 455.510b [Reserved]

§ 455.510c Chloramphenicol-polymyxin ointment.

The requirements for certification and the tests and methods of assay for chloramphenicol-polymyxin ointment are described in § 455.310d.

§ 455.510d Fibrinolysin and desoxyribonuclease, combined (bovine) with chloramphenicol ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Fibrinolysin and desoxyribonuclease, combined (bovine) with chloramphenicol ointment is fibrinolysin, desoxyribonuclease, and chloramphenicol in a suitable and harmless ointment base. It contains a suitable and harmless preservative. Each gram contains 1 unit of fibrinolysin, 666 units of desoxyribonuclease, and 10 milligrams of chloramphenicol. Its chloramphenicol content is satisfactory if it is not less then 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. The chloramphenicol used conforms to the standards prescribed by § 455.10, except paragraph (b)(2) of that section. In addition to the requirements prescribed by this paragraph, the drug satisfies the requirements designated therefor by the Center for Biologics Evaluation and Research, Food and Drug Administration, Department of Health and Human Services.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(b) The batch for potency.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 containers if it is packaged in immediate containers of tin or glass, and a minimum of 20 immediate containers if it is packaged in immediate containers other than tin or glass.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of petroleum ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the aqueous layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the aqueous extractives in a suitable volumetric flask and dilute to volume with distilled water. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5

micrograms of chloramphenicol per milliliter (estimated).

[44 FR 10380, Feb. 20, 1979, as amended at 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985; 55 FR 11585, Mar. 29, 1990]

§ 455.540 Mupirocin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mupirocin ointment is mupirocin in a suitable and harmless ointment base. Each gram of ointment contains 20 milligrams of mupirocin. Its mupirocin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of mupirocin that it is represented to contain. It passes the identity test. The mupirocin used conforms to the standards prescribed by § 455.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The mupirocin used in making the batch for potency, moisture, pH, crystallinity, and identity.

(B) The batch for mupirocin content and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The mupirocin used in making the batch: 10 packages, each containing not less than 300 milligrams.

(B) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Mupirocin content.* Proceed as directed in § 455.40(b)(1), preparing the sample solution and calculating the mupirocin content as follows:

(i) *Sample solution.* Accurately weigh approximately 0.5 gram of ointment and dissolve in 20 milliliters of acetonitrile. Transfer to a 100-milliliter volumetric flask with the aid of pH 6.3 phosphate buffer. Dilute to volume with pH 6.3 phosphate buffer. Mix well. The sample solution contains approximately 100 micrograms of mupirocin per milliliter (estimated).

(ii) *Calculations.* Calculate the mupirocin content in milligrams per gram as follows:

$$\text{Milligrams of mupirocin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

A_s=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

d=Dilution factor of the sample; and

n=Number of grams of sample assayed.

(2) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the mupirocin working standard.

[55 FR 2642, Jan. 26, 1990]

PART 460—ANTIBIOTIC DRUGS INTENDED FOR USE IN LABORATORY DIAGNOSIS OF DISEASE

Subpart A—Susceptibility Discs

Sec.
460.1 Certification procedures for antibiotic susceptibility discs.
460.6 Tests and methods of assay for potency of antibiotic susceptibility discs.
460.11 Certification procedures for antibiotic elution susceptibility discs.
460.15 Streptomycin sulfate discs for use in culture media.
460.16 Rifampin discs for use in culture media.

Subpart B—Susceptibility Powders

460.25 Bacitracin diagnostic sensitivity powder.
460.28 Disodium carbenicillin diagnostic sensitivity powder.
460.33 Clindamycin hydrochloride hydrate sensitivity powder.
460.38 Sodium colistimethate diagnostic sensitivity powder.
460.42 Dihydrostreptomycin sulfate diagnostic sensitivity powder.
460.47 Doxycycline hyclate diagnostic sensitivity powder.
460.55 Lincomycin hydrochloride monohydrate diagnostic sensitivity powder.
460.58 Methacycline hydrochloride diagnostic sensitivity powder.
460.64 Minocycline hydrochloride powder for

microbial susceptibility testing.
460.66 Oleandomycin phosphate diagnostic sensitivity powder.
460.70 Oxytetracycline hydrochloride diagnostic sensitivity powder.
460.75 Potassium penicillin G diagnostic sensitivity powder.
460.79 Polymyxin B sulfate diagnostic sensitivity powder.
460.86 Spectinomycin hydrochloride powder for microbial susceptibility testing.
460.89 Streptomycin sulfate diagnostic sensitivity powder.
460.93 Tetracycline hydrochloride diagnostic sensitivity powder.

Subpart C—Susceptibility Test Panels

460.100 Antimicrobial susceptibility test panels.
460.110 Ampicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.113 Carbenicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.116 Cephalothin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.119 Chloramphenicol concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.122 Clindamycin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.125 Colistin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.128 Erythromycin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.131 Gentamicin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.134 Kanamycin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.137 Methicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.140 Penicillin G concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.146 Tetracycline concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.149 Tobramycin concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.152 Trimethoprim concentrated stock solutions for use in antimicrobial susceptibility test panels.
460.153 Sulfamethoxazole concentrated stock solutions for use in antimicrobial susceptibility test panels.

AUTHORITY: 21 U.S.C. 357.

SOURCE: 39 FR 19181, May 30, 1974, unless otherwise noted.

Subpart A—Susceptibility Discs

§ 460.1 Certification procedures for antibiotic susceptibility discs.

(a) *Standards of identity, strength, quality, and purity.* Antibiotic susceptibility discs are round flat discs that have a diameter of one-fourth inch and are made of clear absorbent paper containing antibiotic compounds. They are capable of absorbing moisture rapidly and the antibiotic is evenly distributed. The thickness is sufficient to assure rigidity and to have permitted the complete absorption of an adequate volume of antibiotic solution (approximately 0.02 milliliter). The identity of each disc is signified either by a color or by means of an identifying sign. The absorbent paper and dye or ink used must not affect either bacterial growth or the antibiotic. Each disc shall have a uniform potency that is equivalent to that contained in a standard disc prepared with one of the following quantities of antibiotic drugs:

Ampicillin: 10 mcg.
Bacitracin: 10 units.
Carbenicillin: 50 mcg.
Cefamandole:30mcg.
Cefoxitin: 30 mcg
Cephalothin: 30 mcg.
Chloramphenicol: 30 mcg.
Clindamycin: 2 mcg.
Colistin: 10 mcg.
Erythromycin: 15 mcg.
Gentamicin: 10 mcg.
Kanamycin: 30 mcg.
Methicillin: 5 mcg.
Neomycin: 30 mcg.
Novobiocin: 30 mcg.
Oleandomycin: 15 mcg.
Penicillin G: 10 units.
Polymyxin B: 300 units.
Rifampin: 5 mcg.
Streptomycin: 10 mcg.
Tetracycline: 30 mcg.
Tobramycin: 10 mcg.
Vancomycin: 30 mcg.

The standard discs used to determine the potency shall be made of paper as described in § 460.6(d). Each antibiotic compound used to impregnate such standard discs shall be equilibrated in terms of the working standard designated by the Commissioner for use in determining the potency or purity of such antibiotic.

(b) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may contain a desiccant, and each may be packaged in combination with containers of suitable discs of drugs other than those described in paragraph (a) of this section. Such other discs shall be suitable only if the manufacturer and packer have submitted to the Commissioner information of the kind described in § 431.17 of this chapter, and such information has been accepted by the Commissioner.

(c) *Labeling.* Each package of discs shall bear on its label or labeling, as hereinafter indicated, the following:

(1) On the outside wrapper or container and the immediate container:

(i) The batch mark.

(ii) The name and potency of each disc in the batch according to the following:

Name of disc	Content of antibiotic in micrograms or units per disc
Ampicillin-class disc	10 mcg. ampicillin.
Bacitracin disc	10 units bacitracin.
Carbenicillin disc	50 mcg. carbenicillin.
Cefamandole disc	30 mcg. cefamandole.
Cefoxitin disc	30 mcg. cefoxitin.
Cephalosporin-class disc	30 mcg. cephalothin.
Chloramphenicol disc	30 mcg. chloramphenicol.
Colistin disc	10 mcg. colistin.
Erythromycin disc	15 mcg. erythromycin.
Gentamicin disc	10 mcg. gentamicin.
Kanamycin disc	30 mcg. kanamycin.
Lincomycin-class disc	2 mcg. clindamycin.
Neomycin disc	30 mcg. neomycin.
Novobiocin disc	30 mcg. novobiocin.
Oleandomycin disc	15 mcg. oleandomycin.
Penicillin-class disc	10 units penicillin G.
Penicillinase-resistant penicillin-class disc.	5 mcg. methicillin.
Polymyxin B disc	300 units polymyxin B.
Rifampin disc	5 mcg. rifampin.
Streptomycin-class disc	10 mcg. streptomycin.
Tetracycline-class disc	30 mcg. tetracycline.
Tobramycin disc	10 mcg. tobramycin.
Vancomycin disc	30 mcg. vancomycin.

(iii) The statement ''Expiration date _____'', the blank being filled in with the date that is 6 months after the month during which the batch was certified, except that the blank may be filled in with a date that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such longer period of time. If it is a packaged combination of discs of two or more drugs, its outside wrapper shall bear only one expiration date, and that date shall be the date that is required for the shortest dated discs contained in the package.

(iv) The statement ''For laboratory use only''.

(2) On the circular or other labeling within or attached to the package, adequate directions for the use of such discs, including the following recommended method:

STANDARDIZED DISC SUSCEPTIBILITY TEST

DIRECTIONS FOR USE

Quantitative methods that require the measurement of zone sizes give the most precise estimates of antibiotic susceptibilities. The following outline describes such a procedure. Minor variations from this procedure may be used if the resulting procedure is standardized according to the results obtained in the laboratory from adequate studies with control cultures.

A. PREPARATION OF CULTURE MEDIUM AND PLATES

1. Melt previously prepared and sterilized Mueller-Hinton agar medium and cool to 45°–50° C.

2. For the purpose of testing certain fastidious organisms such as streptococci and *Haemophilus* species, 5 percent defibrinated human, horse, or sheep blood may be added to the above medium which is ''chocolatized'' when indicated.

3. To prepare the plates, pour the melted medium into Petri dishes on a level surface to a depth of 4 millimeters.

4. Let the medium harden and allow to stand long enough for excess moisture to evaporate. (For this purpose plates may be placed in an incubator at 35°–37° C. for 15–30 minutes or allowed to stand somewhat longer at room temperature.) There should be no moisture droplets on the surface of the medium or on the petri dish covers. The pH of the solidified medium should be 7.2–7.4. Satisfactory plates may be used immediately or refrigerated. Plates may be used as long

as the surface is moist and there is no sign of deterioration.

NOTE: Commercially prepared agar plates meeting the above specifications may be used.

B. PREPARATION OF INOCULUM

1. Select four or five similar colonies.

2. Transfer these colonies (obtained by touching the top of each colony in turn with a wire loop) in turn to a test tube containing about 5 milliliters of a suitable liquid medium such as soybean-casein digest broth, U.S.P.

3. Incubate the tube at 35°–37° C. long enough (2 to 8 hours) to produce an organism suspension with moderate cloudiness. At that point the inoculum density of the suspension should be controlled by diluting it, or a portion of it, with sterile saline to obtain a turbidity equivalent to that of a freshly prepared turbidity standard obtained by adding 0.5 milliliter of 1.175 percent barium chloride dihydrate ($BaCl_2 2H_2O$) solution to 99.5 milliliters of 0.36 N (1.0 percent) sulfuric acid. Other suitable methods for standardizing inoculum density may be used; for example, a photometric method. In some cases it may be possible to get an adequate inoculum density in the tube even without incubation.

NOTE: Extremes in inoculum density should be avoided. Undiluted overnight broth culture should never be used for streaking plates.

C. INOCULATING THE PLATES

1. Dip a sterile cotton swab on a wooden applicator into the properly diluted inoculum. Remove excess inoculum from the swab by rotating it several times with firm pressure on the inside wall of the test tube above the fluid level.

2. Streak the swab over the entire sterile agar surface of a plate. Streaking successively in three different directions is recommended to obtain an even inoculum.

3. Replace the plate top and allow the inoculum to dry for 3 to 5 minutes.

4. Place the susceptibility discs on the inoculated agar surface and with sterile forceps, or needle tip flamed and cooled between each use, gently press down each disc to insure even contact. Space the discs evenly so that they are no closer than 10 to 15 millimeters to the edge of the petri dish and sufficiently separated from each other to avoid overlapping zones of inhibition. (Spacing may be accomplished by using a disc dispenser or by putting the plate over a pattern to guide the placement of discs.) Within 30 minutes, place the plate in an incubator under aerobic conditions at a constant temperature in the range of 35°–37° C.

5. Read the plate after overnight incubation or, if rapid results are desired, the diameters of the zone of inhibition may be readable after 6 to 8 hours incubation. In the latter case, the results should be confirmed by also reading the results after overnight incubation.

NOTE: Microbial growth on the plate should be just or almost confluent. If only isolated colonies are present the inoculum was too light and the test should be repeated.

Modifications of the inoculation procedure described in 1–3 above, such as the use of the agar overlay method described in Barry, A. L., Garcia, F., and Thrupp, L. D.: ''An Improved Single-disk Method for Testing the Antibiotic Susceptibility of Rapidly-growing Pathogens.'' Amer. J. Clin. Pathol. 53:149–58, 1970,* a copy of which is on file with the Office of the Federal Register, may be used if the procedure is standardized to produce results with the control cultures that are equivalent to those obtained with the recommended cotton swab streak method.

D. READING THE PLATES

Measure and record the diameter of each zone (including the diameter of the disc) to the closest millimeter, reading to the point of complete inhibition as judged by the unaided eye. Preferably, read from the underside of the plate without removing the cover, using a ruler, calipers, transparent plastic gage, or other device. A mechanical zone reader may be used. If blood agar is used, measure the zones from the surface with the cover removed from the plate.

E. INTERPRETATION OF ZONE SIZES

Interpret the susceptibility according to the following table:

Antibiotic	Disc content	Diameter (millimeters) of zone of inhibition		
		Resistant	Intermediate	Susceptible
Ampicillin[1] when testing gram-negative microorganisms and enterococci.	10 mcg	11 or less	12–13	14 or more.
Ampicillin[1] when testing staphylococci and penicillin G—susceptible micro-organisms.	10 mcg	20 or less	21–28	29 or more.
Ampicillin[1] when testing *Haemophilus* species	10 mcg	19 or less		20 or more.

*Copies may be obtained from: J. B. Lippincott Company, Attn: Circulation Manager, East Washington Square, Philadelphia, PA 19105.

Antibiotic	Disc content	Diameter (millimeters) of zone of inhibition		
		Resistant	Intermediate	Susceptible
Bacitracin	10 units	8 or less	9–12	13 or more.
Carbenicillin when testing *Proteus* species and *Escherichia coli*	50 mcg	17 or less	18–22	23 or more.
Carbenicillin when testing *Pseudomonas aeruginosa*	50 mcg	12 or less	13–14	15 or more.
Cefamandole [12]	30mcg	14 or less	16–17	18 or more.
Cefoxitin [11]	30 mcg	14 or less	15–17	18 or more.[11]
Cephalothin when reporting susceptibility to cephalothin, cephaloridine, and cephalexin.	30 mcg	14 or less	15–17	18 or more.[2]
Cephalothin when reporting susceptibility to cephaloglycin	30 mcg	14 or less		15 or more.
Chloramphenicol	30 mcg	12 or less	13–17	18 or more.
Clindamycin [3] when reporting susceptibility to clindamycin	2 mcg	14 or less	15–16	17 or more.
Clindamycin when reporting susceptibility to lincomycin	2 mcg	16 or less	17–20	21 or more.
Colistin	10 mcg	8 or less	9–10	11 or more.
Erythromycin	15 mcg	13 or less	14–17	18 or more.
Gentamicin	10 mcg	12 or less		13 or more.
Kanamycin	30 mcg	13 or less	14–17	18 or more.
Methicillin [5]	5 mcg	9 or less	10–13	14 or more.
Neomycin	30 mcg	12 or less	13–16	17 or more.
Novobiocin	30 mcg	17 or less	18–21	22 or more.[6]
Oleandomycin [7]	15 mcg	11 or less	12–16	17 or more.
Penicillin G when testing staphylococci [8]	10 units	20 or less	21–28	29 or more.
Penicillin G when testing other micro-organisms [8]	10 units	11 or less	[9] 12–21	22 or more.
Polymyxin B	300 units	8 or less	9–11	12 or more.[4]
Rifampin when testing *Neisseria meningitidis* susceptibility only	5 mcg	24 or less		25 or more.
Streptomycin	10 mcg	11 or less	12–14	15 or more.
Tetracycline [10]	30 mcg	14 or less	15–18	19 or more.
Tobramycin	10 mcg	11 or less	12–13	14 or more.
Vancomycin	30 mcg	9 or less	10–11	12 or more.

[1] The ampicillin disc is used for testing susceptibility to both ampicillin and hetacillin.
[2] Staphylococci exhibiting resistance to the penicillinase-resistant penicillin class discs should be reported as resistant to cephalosporin class antibiotics. The 30 mcg. cephalothin disc cannot be relied upon to detect resistance of methicillin-resistant staphylococci to cephalosporin class antibiotics.
[3] The clindamycin disc is used for testing susceptibility to both clindamycin and lincomycin.
[4] Colistin and polymyxin B diffuse poorly in agar and the accuracy of the diffusion method is thus less than with other antibiotics. Resistance is always significant but when treatment of systemic infections due to susceptible strains is considered, it is wise to confirm the results of a diffusion test with a dilution method.
[5] The methicillin disc is used for testing susceptibility to all penicillinase-resistant penicillins; that is, methicillin, cloxacillin, dicloxacillin, oxacillin, and nafcillin.
[6] Not applicable to medium that contains blood.
[7] The oleandomycin disc is used for testing susceptibility to oleandomycin and troleandomycin.
[8] The penicillin G disc is used for testing susceptibility to all penicillinase-susceptible penicillins except ampicillin and carbenicillin; that is, penicillin G, phenoxymethyl penicillin, and phenethicillin.
[9] This category includes some organisms such as enterococci and gram-negative bacilli that may cause systemic infections treatable with high doses of penicillin G. Such organisms should only be reported susceptible to penicillin G and not to phenoxymethyl penicillin or phenethicillin.
[10] The tetracycline disc is used for testing susceptibility to all tetracyclines; that is, chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, rolitetracycline, minocycline, and tetracycline.
[11] The cefoxitin disc should not be used for testing susceptibility of other cephalosporins.
[12] The cefamandole disc should not be used for testing susceptibility of other cephalosporins.

F. REFERENCE ORGANISMS

1. Maintain stock cultures of *Staphylococcus aureus* (ATCC 25923) [1] and *Escherichia coli* (ATCC 25922). [1]

2. Test these reference organisms daily by the above procedure using antibiotic discs representative of those to be used in the testing of clinical isolates.

3. The individual values of zone sizes for the control organisms can be expected to fall in the ranges indicated in the following table:

Antibiotic	Disc content	Individual tests		
		Zone diameter in millimeters		Permitted millimeter difference ATCC 25923–ATCC 25922
		With *S. aureus* ATTC 25923 [1]	With *E. coli* ATCC 25922 [1]	
Ampicillin	10 mcg	24–35	15–20	7–17
Bacitracin	10 units	17–22		
Cefamandole	30 mcg	28–34	24–31	−1–6.8
Cefoxitin	30 mcg	24–32	25–30	3–4
Cephalothin	30 mcg	25–37	18–23	5–16

Antibiotic	Disc content	Individual tests		
		Zone diameter in millimeters		Permitted millimeter difference ATCC 25923–ATCC 25922
		With *S. aureus* ATTC 25923 [1]	With *E. coli* ATCC 25922 [1]	
Chloramphenicol	30 mcg	19–26	21–27	−4–1
Clindamycin	2 mcg	23–29	.....	.....
Colistin	10 mcg	.....	11–15	.....
Erythromycin	15 mcg	22–30	8–14	10–19
Gentamicin	10 mcg	19–27	19–26	−2–3
Kanamycin	30 mcg	19–26	17–25	−1–4
Methicillin	5 mcg	17–22	.....	.....
Neomycin	30 mcg	18–26	17–23	0–3
Novobiocin	30 mcg	22–31	.....	.....
Oleandomycin	15 mcg	19–28	.....	.....
Penicillin G	10 units	26–37	.....	.....
Polymyxin B	300 units	7–13	12–16	−7–−2
Streptomycin	10 mcg	14–22	12–20	−1–5
Tobramycin	10 mcg	19–29	18–26	.....
Tetracycline	30 mcg	19–28	18–25	0–6
Vancomycin	30 mcg	15–19	.....	.....

[1] Available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, Md. 20852.

G. LIMITATIONS OF THE METHOD

The method of interpretation described in E above applies to rapidly growing pathogens and should not be applied to slowly growing organisms. The latter show larger zones of inhibition than those given in the table. Susceptibility of gonococci to penicillin, and of slow-growing strains, e.g., *Bacteroides* species and fastidious anaerobes to any antibiotic, should be determined by the broth-dilution or agar-dilution method unless specifically standardized diffusion tests are used.

(d) *Requests for certification; samples.* (1) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of antibiotic susceptibility discs shall submit with his request a statement showing the batch mark, the number of packages of each size in such batch, and, unless it was previously submitted, the date on which the latest assay of the antibiotic used in making such batch was completed, the potency of each disc, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug comprising such batch was completed, and a statement that each ingredient used in making the batch conforms to the requirements prescribed therefor by this section.

(2) Such person shall submit in connection with his request results of the tests and assays made by him on an accurately representative sample of the batch for potency.

(3) Such person shall submit in connection with his request an accurately representative sample of the batch consisting of one disc for each 5,000 discs in the batch, but in no case less than 36 discs collected by taking single discs at intervals throughout the entire time of packaging the batch so that the quantities packaged during the intervals are approximately equal.

[39 FR 19181, May 30, 1974, as amended at 41 FR 7093, Feb. 17, 1976; 41 FR 35061, Aug. 19, 1976; 44 FR 10376, Feb. 20, 1979; 44 FR 20666, Apr. 6, 1979]

§ 460.6 Tests and methods of assay for potency of antibiotic susceptibility discs.

(a) *Culture media.* Use ingredients that conform to the standards prescribed by the United States Pharmacopeia or The National Formulary. In lieu of preparing the media from the individual ingredients, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the specified individual ingredients is permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(1) *Medium A:*

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.

Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) *Medium B.* Same as medium A, except that it also contains 300 milligrams of hydrated manganese sulfate per liter.

(3) *Medium C.* Same as medium A except that the final pH is adjusted from 7.9 to 8.1 after sterilization.

(4) *Medium D:*

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Disilled water, q.s	1,000.0 ml.

pH 7.0 after sterilization

(5) *Medium E:*

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(6) *Medium F:*

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Agar	20.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.3 after sterilization.

(7) *Medium G.* Same as medium F except for the following:

Agar	12.0 gm.
Polysorbate 80 (Sterile)	10.0 gm.

Add polysorbate 80 after boiling.

(8) *Medium H:*

Peptone	9.4 gm.
Yeast extract	4.7 gm.
Beef extract	2.4 gm.
Sodium chloride	10.0 gm.
Dextrose	10.0 gm.
Agar	23.5 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.0 to 6.2 after sterilization.

(9) *Medium I:*

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.6 after sterilization.

(10) *Medium J:*

Pancreatic digest of casein	15.0 gm.
Papaic digest of soybean	5.0 gm.
Sodium chloride	5.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.3 after sterilization.

(11) *Medium K:*

Pancreatic digest of casein	17.0 gm.
Papaic digest of soybean	3.0 gm.
Sodium chloride	5.0 gm.
Dipotassium phosphate	2.5 gm.
Dextrose	2.5 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.3 after sterilization.

(12) *Medium L:*

Agar agar	15 gm.
Distilled water, q.s	1,000.0 ml.

(13) *Medium M:*

Beef, inclusion from	300 gm.
Acid hydrolysate of casein	17.5 gm.
Soluble starch	1.5 gm.
Agar	15 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.4 after sterilization.

(14) *Medium N:*

Infusion from beef	300.0 gm.
Acid hydrolysate of casein	17.5 gm.
Starch	1.5 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.4 after sterilization.

(15) *Medium O:*

Calf brains, infusion from	200.0 gm.
Beef heart, infusion from	250.0 gm.
Pancreatic digest of gelatin	10.0 gm.
Dextrose	2.0 gm.
Sodium chloride	5.0 gm.
Sodium phosphate dibasic (Na_2HPO_4)	2.5 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.4 after sterilization.

(16) *Medium P.* Same as medium J with 5 percent defibrinated sheep blood added.

(17) *Medium Q:*

Pancreatic digest of gelatin	17.0 gm.
Pancreatic digest of casein plus equal part of peptic digest of animal tissues	3.0 gm.
Lactose	10.0 gm.
Bile salts mixture	1.5 gm.
Sodium chloride	5.0 gm.
Agar	13.5 gm.
Neutral red	0.03 gm.
Crystal violet	0.001 gm.
Distilled water, q.s	1,000.0 ml.

pH 7.1 after sterilization.

(b) *Preparation of test organism suspensions*—(1) *Suspension 1. Staphylococcus aureus* (ATCC 6538P)[1] is maintained and

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

grown on medium A. Wash the organisms from an agar slant, incubated for 24 hours at 32° C. to 35° C., with 3.0 milliliters of sterile sodium chloride solution onto the agar surface of a Roux bottle containing 300 milliliters of medium A. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate 24 hours at 32° C. to 35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this stock suspension by determining the dilution that will permit 20 percent light transmission. Store the stock suspension in the refrigerator (1 week) and use the indicated dilution prepared daily.

(2) *Suspension 2.* Follow the procedure described for suspension 1, except standardize the bulk suspension so that a 1:10 dilution in saline solution gives 20 percent light transmission. In this case, the bulk suspension, and not the 1:10 dilution of it, is used for the inoculum.

(3) *Suspension 3.* The test organism is *Staphylococcus aureus* (ATCC 13150).[1] Follow the procedure described for suspension 1, but determine how much the bulk suspension should be diluted to obtain a suspension permitting 80 percent light transmission. Use the indicated dilution prepared daily for the inoculum for the plates.

(4) *Suspension 4. Sarcina lutea* (ATCC 9341)[1] is maintained on agar slants of medium A and transferred to fresh slants approximately every 2 weeks. This culture is incubated overnight at 26° C., and then stored in the refrigerator. Prepare an inoculum for the plates as follows: Streak an agar slant heavily with the test organism and incubate for 24 hours at 26° C. Wash the growth from the slant with 3 milliliters to 4 milliliters of medium D, and transfer to the surface of a Roux bottle containing 300 milliliters of medium A. Spread the suspension evenly over the entire surface with the aid of sterile glass beads. Incubate for 24 hours at 26° C. Wash the growth from the agar surface with 15 milliliters of medium D. If an aliquot of this bulk suspension when diluted 1:10 with medium D gives 10 percent light transmission, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension when diluted 1:10 will give the desired 10 percent light transmission. The adjusted bulk suspension only, and not the 1:10 dilution of it, is used in preparing the inoculum. Store the stock suspension in the refrigerator and use for 2 weeks.

(5) *Suspension 5. Bacillus subtilis* (ATCC 6633)[1] is maintained on agar medium A and transferred to a fresh slant every month. To prepare the spore suspension, inoculate a fresh slant of agar medium A with the test organism and incubate at 37° C. for 16 hours to 24 hours. Wash the culture from the slant with 3 milliliters of sterile sodium chloride solution onto the surface of a Roux bottle containing 300 milliliters of agar medium B. Incubate for 5 days at 37° C. Suspend the growth in 50 milliliters of sterile saline solution, centrifuge, and decant the supernatant liquid. Reconstitute the sediment and heat-shock the suspension by heating for 30 minutes at 70° C. Store the spore suspension in the refrigerator. It may be kept several months. Light transmission is not used for standardization.

(6) *Suspension 6. Staphylococcus epidermidis* (ATCC 12228)[1] is maintained on medium A and transferred to a fresh slant once a week. Inoculate a fresh slant of medium A with the test organism and incubate at 32° C. to 35° C. for 24 hours. Wash the culture from the slant with 3 milliliters of sterile sodium chloride solution onto the surface of a Roux bottle containing 300 milliliters of medium A. Incubate at 32° C. to 35° C. for 24 hours. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize this stock suspension by determining the dilution that will give 80 percent light transmission. Store the stock suspension in the refrigerator (1 week) and use the indicated dilution prepared daily for the inoculum for the plates.

(7) *Suspension 7. Bordetella bronchiseptica* (ATCC 4617)[1] is maintained on medium F and transferred to a fresh slant every 2 weeks. To prepare a stock suspension inoculate a fresh slant of medium F and incubate at 37° C. for 16 hours to 24 hours. Wash the

culture from this slant with 3 milliliters of sterile distilled water onto the surface of a Roux bottle containing 300 milliliters of medium F, and incubate 24 hours at 37° C. Wash off the growth with 50 milliliters of sterile distilled water and standardize the resulting stock suspension by determining the dilution that will give 50 percent light transmission. Store the stock suspension in the refrigerator (2 weeks), and use the indicated dilution prepared daily for the inoculum for the plates.

(8) *Suspension 8. Saccharomyces cerevisiae* (ATCC 9763)[1] is maintained on slants of medium H and transferred once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Wash the organism from a freshly incubated agar slant with 3 milliliters of sterile saline solution onto the agar surface in a Roux bottle containing 300 milliliters of medium H. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. and then wash the resulting growth from the agar surface with about 25 milliliters of sterile saline solution. Store the suspension in the refrigerator and use for 1 month.

(9) *Suspension 9.* Follow the procedure described for suspension 1, except determine how much the bulk suspension should be diluted to obtain a suspension permitting 80 percent light transmission. Use the indicated dilution, prepared daily, for the inoculum for the plates.

(10) *Suspension 10: Klebsiella pneumoniae* (ATCC 10031),[1] noncapsulated, is maintained on medium A and transferred to a fresh slant once a week. Inoculate a fresh slant of medium A with the test organism and incubate overnight at 32° C.–35° C. Wash the culture from the slant with 3 milliliters of sterilized U.S.P. saline T.S. onto the surface of a Roux bottle containing 300 milliliters of medium A. Incubate at 32° C.–35° C. for 24 hours. Wash the resulting growth from the agar surface with about 50 milliliters of sterilized U.S.P. saline T.S. If an aliquot of this bulk suspension when diluted 1:9 with saline solution gives 40 percent light transmission, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution so that an aliquot of the adjusted suspension when diluted 1:9 will give the desired 40 percent light transmission. The adjusted bulk suspension (not the 1:9 dilution) is used in preparing the inoculum. Store the suspension in the refrigerator and use for no more than 1 week.

(11) *Suspension 11 Streptococcus fecalis* (ATCC 14506)[1] is maintained on medium E and transferred to a fresh agar slant once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Transfer from a freshly incubated agar slant to a tube containing 10 milliliters of culture medium described in § 147.3(b)(1). Incubate the broth culture for 16 to 18 hours at 37° C. and store in the refrigerator. This culture may be used for no more than 1 week.

The light transmission values referred to in this paragraph were determined with a Lumetron Model 400–A photoelectric colorimeter at a wavelength of 650 millimicrons. If other instruments are used, different light transmission readings will probably be obtained. The values given are to be used as guides in this paragraph.

(12) *Suspension 12. Pseudomonas aeruginosa* (ATCC 25619)[1] is maintained and grown on medium J and transferred to a fresh agar slant once a week. Inoculate a fresh slant of medium J with the test organism and incubate at 37° C. for 24 hours. Transfer the culture from this slant with sterile glass beads onto the agar surface of a Roux bottle containing 300 milliliters of medium J. Spread the organisms over the entire agar surface with the aid of the glass beads. Incubate 24 hours at 37° C. Wash the resulting growth from the agar surface with about 30 milliliters of medium K. Do not standardize the suspension. Store the stock suspension under refrigeration and use for 2 weeks.

(13) *Suspension 13. Escherichia coli* (ATCC 29214)[1] is maintained and grown on medium M. Wash the organisms from an agar slant, incubated for 24 hours at 37° C, with 3 milliliters of

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

sterilized U.S.P. saline T.S. onto the surface of a Roux bottle containing 250 milliliters of medium M. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C and then wash the resulting growth from the agar surface with 50 milliliters of sterilized U.S.P. saline T.S. Store the suspension in the refrigerator and use for 2 weeks.

(14) *Suspension 14.* The test organism is *Staphylococcus aureus* (ATCC 29213).

(i) *Stock culture.* Transfer a lyophilized culture into medium K in a sterile container and incubate at 37° C for 24 hours. Streak the culture onto the solidified agar surface of a plate containing medium P and incubate the plate at 37° C for 24 hours. Transfer 5 to 10 colonies into 3 milliliters of medium O in a sterile container and incubate at 37° C for 24 hours. Add 3 milliliters of sterile glycerol or 3 milliliters of sterile rabbit serum to the broth culture, mix well and pour the contents into a sterile flask containing a layer of sterile glass beads. Rotate the flask to coat the beads with the culture mixture and aseptically aspirate all the excess liquid from the flask. Store the flask containing the coated glass beads at −20° C to −70° C.

(ii) *Test suspension.* Aseptically add a coated glass bead to 0.5 milliliter of medium O and incubate at 37° C for 24 hours. Streak the culture onto the solidified agar surface of a plate containing medium P and incubate at 37° C for 24 hours. The streak plate may be used for 1 week if kept under refrigeration. On the day of test, transfer 4 to 10 colonies to a sterile tube containing 0.5 milliliter of medium O and incubate at 37° C for 4 to 6 hours. Pipet 0.05 milliliter of the test suspension into a screw-topped tube containing 25 milliliters of sterile distilled water and 0.005 milliliter of sterile polysorbate 80 and mix well (do not shake). Use this test culture suspension as the daily inoculum source.

(15) *Suspension 15.* The test organism is *Escherichia coli* (ATCC 25922).1 Follow the procedure described for suspension 14 in paragraph (b)(14) of this section, except under paragraph (b)(14)(ii) of this section use medium Q in place of medium P.[1]

(16) *Suspension 16.* The test organism is *Streptococcus faecalis* (ATCC 29212)[1]. Follow the procedure described for suspension 14 in paragraph (b)(14) of this section.

(17) *Suspension 17.* The test organism is *Pseudomonas aeruginosa* (ATCC 27853)[1]. Follow the procedure described for suspension 14 in paragraph (b)(14) of this section, except under paragraph (b)(14)(ii) of this section use medium Q in place of medium P.

(18) *Suspension 18.* The test organism is *Staphyloccocus aureas* (ATCC 29247)[1]. Follow the procedure described for suspension 14 in paragraph (b)(14) of this section.

(19) *Suspension 19.* The test organism is *Enterobacter clocacae* (ATCC 29249)[1]. Follow the procedure described for suspension 14 in paragraph (b)(14) of this section.

(20) *Suspension 20.* The test organism is *Pseudomonas aeruginosa* (ATCC 29248)[1]. Follow the procedure described for suspension 14 in paragraph (b)(14) of this section, except under paragraph (b)(14)(ii) of this section use medium Q in place of medium P.

(c) *Preparation of plates*—(1) *Baselayer.* Depending on the particular antibiotic in the discs to be tested, add 42 milliliters of the appropriate medium prescribed in paragraph (c)(3) of this section to each Petri dish (20 millimeters x 150 millimeters) and allow to harden on a flat, level surface and dry slightly by raising the tops on one side.

(2) *Seed layer.* Add the appropriate amount of inoculum, as prescribed by paragraph (c)(3) of this section, to the seed agar which has been melted and cooled to 48° C. Swirl the flasks to obtain a homogeneous suspension. Add 8 milliliters of the appropriate seed agar, as specified in paragraph (c)(3) of this section, to each plate, spread evenly over the hardened base layer, and allow to harden and dry on a flat level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the surface of the plates.

[1] Available from American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852.

(3) *Inoculum and media to be used.* Depending on the particular antibiotic in the disc to be tested, select from the following table the inoculum and media to be used:

Antibiotic	Volume of suspension added to each 100 ml. of seed agar used for test	Suspension number	Medium	
			Base layer	Seed layer
	Ml.			
Ampicillin	1.0	3	E	A
Bacitracin	1.0	3	E	A
Carbenicillin	3.0	12	F	G
Cefamandole (lithium)	1.0	10	E	A
Cefoxitin (sodium)	1.0	10	E	A
Cephaloglycin (dihydrate)	1.0	10	E	A
Cephaloridine	1.0	10	E	A
Cephalothin	1.0	10	E	A
Chloramphenicol	4.0	4	E	A
Clindamycin	2.0	2	A	I
Colistin (sulfate)	1.0	7	F	G
Erythromycin	2.0	11	C	C
Gentamicin (sulfate)	0.5	3	C	C
Kanamycin (sulfate)	1.0	9	E	A
Methicillin	1.0	3	E	A
Neomycin (sulfate)	2.5	6	C	C
Novobiocin (sodium)	4.0	5	E	A
Oleandomycin (phosphate)	2.0	3	E	A
Penicillin G	1.0	3	E	A
Polymyxin B (sulfate)	1.0	7	F	G
Rifampin	1.0	5	E	A
Rifampin discs for use in culture media	0.5	13	A	L
Streptomycin (sulfate)	3.0	1	C	C
Tetracycline (hydrochloride)	1.5	1	E	A
Tobramycin	0.5	3	C	C
Vancomycin	1.0	6	C	C

(d) *Preparation of control discs.* Use round, blank discs having a diameter of ¼-inch made of clear-white paper weighing 30 milligrams ±4 milligrams per square centimeter, and which will absorb 2.5 to 3.0 times its own weight of distilled water. The paper shall contain no material that either enhances or inhibits the activity of any antibacterial agent impregnated thereon. In addition, the paper shall contain no materials which will affect the pH of any solvent placed on it or buffer any solution placed on it. The following methods shall be used to determine the suitability in this regard of any paper proposed for this use: Weigh 2 grams of paper or paper discs into a clean, glass-stoppered, 250-milliliter flask. Add 30 milliliters of freshly boiled and cooled distilled water (the pH of which has been determined). Stopper and shake vigorously for 1 hour on a shaking machine. Filter through a medium-porosity sintered glass filter. Determine the pH of the filtrate. Take the two 10-milliliter aliquots. To one add 0.05 milliliter of 0.01*N* HCl. To the second aliquot add 0.05 milliliter of 0.01 *N* NaOH. Determine the pH of each solution. The paper shall be satisfactory for use, if (1) the pH of the paper filtrate was not more than ±0.3 pH units different from the pH of the distilled water used; (2) the pH of the acidified aliquot was lowered by at least 1.0 pH units; (3) the pH of the alkalinized aliquot was raised by at least 1.5 pH units. Place blank discs on aluminum or stainless steel wire mesh which is supported in a manner to allow circulation of air above and below the discs. Prepare the desired number of discs for each point on the standard curve by accurately adding 0.02-milliliter-increments of the appropriate standard stock solution to each disc, using a suitable pipette. Dry discs in circulating air or under vacuum. Discs may be stored for 2 weeks in a

desiccator under refrigeration. Depending on the antibiotic contained in the sample to be tested, prepare the stock solutions for the standard discs by dissolving an accurately weighed quantity of the working standard in the solvent indicated to obtain stock solutions that will contain the following concentrations required for the standard discs:

Antibiotic	Solvent	Standard curve (antibiotic concentration per disc)
Ampicillin	Water	1.3.2.4, 4.4, 8.1, 15.0µg.
Bacitracin	.....do	1.3, 2.4, 4.4, 8.1, 15.0 units.
Carbenicillin	Methyl alcohol	25.0, 35.5, 50.0, 70.7, 100µg.
Cefamandole (lithium)	50 percent methyl alcohol	5, 30, and 60 µgg.
Cefoxitin (sodium)	50 percent methyl alcohol	5, 30, 60 µg.
Cephalothin	50 percent methyl alcohol	15.0, 21.2, 30.3, 42.4, 60.0µg.
Chloramphenicol	.....do	3.3, 6.3, 12.2, 23.4, 45.0µg.
Clindamycin	.....do	1.0, 1.41, 2.0, 2.82, 4.0µg.
Colistin (sulfate)	Water	1.3, 2.4, 4.4, 8.1, 15.0µg.
Erythromycin	Methyl alcohol	1.3, 2.7, 5.4, 11.0, 2250µg.
Gentamicin (sulfate)	Water	5, 7.1, 10, 14.1, 20µg.
Kanamycin (sulfate)	.....do	3.3, 6.3, 12.2, 23.4, 45µg.
Methicillin	.....do	1.3, 2.4, 4.4, 8.1, 15.0µg.
Neomycin (sulfate)	.....do	3.3, 6.3, 12.2, 23.4, 45.0µg.
Novobiocin (sodium)	.....do	3.3, 6.3, 12.2, 23.4, 45.0µg.
Oleandomycin (phosphate)	.....do	1.3, 2.7, 5.4, 11.0, 22.5µg.
Penicillin G	.....do	1.3, 2.4, 4.4, 8.1, 15.0 units.
Polymyxin B (sulfate)	.....do	33, 63, 122, 234, 450 units.
Rifampin	Methyl alcohol	3.0, 6.0, 12.0, 24.0, 48.0µg.
Rifampin discs for use in culture media	.....do	12.5, 25, 50µg.
Streptomycin (sulfate)	Water	1.3, 2.4, 4.4, 8.1, 15.0µg.
Tetracycline (hydrochloride)	Methyl alcohol	3.3, 6.3, 12.2, 23.4, 45.0µg.
Tobramycin	Water	5, 10, and 20µg.
Vancomycin (hydrochloride)	.....do	3.3, 6.3, 12.2, 23.4, 45.0µg.

(e) *Assay*—(1) *Individual discs one-fourth inch in diameters*—(i) *Standard curves with five antibiotic concentrations.* On each of five plates prepared as directed in paragraph (c) of this section, place the five control discs for the standard curve and two discs from each batch to be tested. The control discs for the standard curve and the sample discs are placed on the plates in a random arrangement, with no discs being closer than 24 millimeters (on centers) to another disc. Discs are placed on the plates with the aid of forceps within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. Incubate the plates overnight at 32° C to 35° C, except if it is cephalothin, colistin, novobiocin, polymyxin, or viomycin, the incubation temperature is 37° C. After incubation, measure the diameter of each circle of inhibition, using calipers or a measuring device of comparable accuracy. Average the three zone sizes for each of the five standard-curve concentrations and plot the mean sizes on the arithmetic scale of semilogarithmic graph paper with the antibiotic concentrations on the logarithmic scale. Use the following equation to calculate the best straight line:

$$L=(3a+2b+c-e)/(5),$$
$$H=(3e+2d+c-a)/(5),$$

where:

L=the calculated zone size of the low concentration;

H=the calculated zone size of the high concentration;

a, b, c, d, e=the observed average zone sizes for each respective concentration, a being that for the lowest concentration.

Plot the values obtained for L and H and connect these two points with a straight line. Average the six sample zone sizes and read the corresponding antibiotic concentration of this mean from the standard curve. This is the potency obtained for a single assay. Perform two or more replicate assays on each of 2 days. The average of all assays is the potency of the sample disc.

(ii) *Standard curves with three antibiotic concentrations.* On each of three plates prepared as directed in paragraph (c) of this section, place the three control discs for the standard

curve and two discs from each batch to be tested. The control discs for the standard curve and the sample discs are placed on the plates in a random arrangement, with no discs being closer than 24 millimeters (on centers) to any other discs. Discs are placed on the plates with the aid of forceps within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. Incubate the plates overnight at 32° C to 35° C, except if it is rifampin discs for use in culture media, the incubation temperature is 37° C. After incubation, measure the diameter of each circle of inhibition, using calipers or a measuring device of comparable accuracy. Average the three zone sizes for each of the three standard curve concentrations and plot the mean sizes on the arithmetic scale of semilogarithmic graph paper with the antibiotic concentrations on the logarithmic scale. Using the following equation to calculate the best straight line:

$$L=(5a+2b-c)/(6),$$
$$H=(5c+2b-a)/(6),$$

where:

L=calculated zone diameter of the lowest concentration of the standard curve;

H=calculated zone diameter of the highest concentration of the standard curve;

a, b, c=observed average zone sizes for each respective concentration, a being that for the lowest concentration.

Plot the values obtained for L and H and connect these two points with a straight line. Average the six sample zone sizes and read the corresponding antibiotic concentration of this mean from the standard curve. This is the potency obtained for a single assay. Perform two or more replicate assays on each of 2 days. The average of all assays is the potency of the sample disc.

(2) *Discs one-fourth inch in diameter attached to rings, spokes, or other devices.* Remove or cut the disc from the device, including a small portion of the device to which it is attached, before testing, and proceed as directed in paragraph (e)(1) of this section.

(3) *Individual discs with diameters larger than one-fourth inch but no larger than three-eighths inch for use in impregnating culture media.* Proceed as directed in paragraph (e)(1) of this section, except instead of measuring the diameters of the zones of inhibition, measure the widths of the zones from any edge of the sample discs and the standard discs. The results obtained are multiplied by the factor 2 for determining whether the discs meet the requirements for uniformity prescribed by paragraph (f) of this section.

(f) The potency is satisfactory if the result obtained is not less than 67 percent and not more than 150 percent of that represented. The batch has a uniform potency if on the first or second test of six discs each, the diameter of the largest zone of inhibition is not more than 2.5 millimeters larger than the smallest zone, or if the number of zones that fall outside this range in three or more consecutive tests is not more than 10 percent of the total number of discs tested.

[39 FR 19181, May 30, 1974, as amended at 41 FR 7094, Feb. 17, 1976; 41 FR 53476, Dec. 7, 1976; 43 FR 9792, Mar. 10, 1978; 43 FR 12858, Mar. 28, 1978; 44 FR 10376, Feb. 20, 1979; 44 FR 20667, Apr. 6, 1979]

§ 460.11 Certification procedures for antibiotic elution susceptibility discs.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Antibiotic elution susceptibility discs are round flat discs that have a diameter of 6.35 millimeters (¼ inch) and are made of absorbent paper containing antibiotic compounds. The identity of each disc is signified by means of an identifying sign. The absorbent paper and dye or ink used must not affect either bacterial growth or the antibiotic. Each disc shall have a potency that is equivalent to that contained in a standard disc prepared with the following quantities of antibiotic drugs:

Ampicillin: 0.22 mcg.
Ampicillin: 4.5 mcg.
Bacitracin: 18.0 units.
Carbenicillin: 120.0 mcg.
Cephalothin: 15.0 mcg.
Chloramphenicol: 4.0 mcg.
Clindamycin: 2.0 mcg.
Colistin: 13.0 mcg.
Doxycycline: 0.5 mcg.
Doxycycline: 1.6 mcg.
Erythromycin: 2.5 mcg.
Gentamicin: 9.0 mcg.

Kanamycin: 22.0 mcg.
Methicillin: 5.0 mcg.
Neomycin: 24.0 mcg.
Novobiocin: 2.5 mcg.
Oleandomycin: 6.0 mcg.
Penicillin: 0.2 unit.
Streptomycin: 20.0 mcg.
Tetracycline: 0.5 mcg.
Tetracycline: 1.2 mcg.
Tobramycin: 10.0 mcg.
Vancomycin: 10.0 mcg.

The standard discs used to determine the potency shall be made of paper as described in § 460.6(d). Each antibiotic compound used to impregnate such standard discs shall be equilibrated in terms of the working standard designated by the Commissioner for use in determining the potency or purity of such antibiotic.

(2) *Packaging.* The immediate container shall be a tight container as defined by the U.S.P. and shall be of such composition as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused that are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded. Each immediate container may contain a desiccant, and each may be packaged in combination with containers of suitable discs of drugs other than those described in paragraph (a)(1) of this section. Such other discs shall be suitable only if the manufacturer and packer have submitted to the Commissioner information of the kind described in § 431.17 of this chapter, and such information has been accepted by the Commissioner.

(3) *Labeling.* Each package of discs shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container:

(*a*) The batch mark.

(*b*) The name and potency of each disc in the batch according to the following:

Name of disc and content of antibiotic in micrograms or units per disc

Ampicillin elution disc, 0.22 mcg.
Ampicillin elution disc, 4.5 mcg.
Bacitracin elution disc, 18.0 units.
Carbenicillin elution disc, 120.0 mcg.
Cephalothin elution disc, 15.0 mcg.
Chloramphenicol elution disc, 4.0 mcg.
Clindamycin elution disc, 2.0 mcg.
Colistin elution disc, 13.0 mcg.
Doxycycline elution disc, 0.5 mcg.
Doxycycline elution disc, 1.6 mcg.
Erythromycin elution disc, 2.5 mcg.
Gentamicin elution disc, 9.0 mcg.
Kanamycin elution disc, 22.0 mcg.
Neomycin elution disc, 24.0 mcg.
Novobiocin elution disc, 2.5 mcg.
Oleandomycin elution disc, 6.0 mcg.
Penicillin elution disc, 0.2 unit.
Methicillin elution disc, 5.0 mcg.
Streptomycin elution disc, 20.0 mcg.
Tetracycline elution disc, 0.5 mcg.
Tetracycline elution disc, 1.2 mcg.
Tobramycin elution disc, 10.0 mcg.
Vancomycin elution disc, 10.0 mcg.

(*c*) The statement ''Expiration date _____'', the blank being filled in with the date that is 6 months after the month during which the batch was certified, except that the blank may be filled in with a date that is 12, 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drugs as prepared by that person are stable for such longer period of time. If it is a packaged combination of discs of two or more drugs, its outside wrapper shall bear only one expiration date, and that date shall be the date that is required for the shortest dated discs contained in the package.

(*d*) The statement ''FOR IN VITRO DIAGNOSTIC USE''.

(ii) On the circular or other labeling within or attached to the package, adequate directions for the use of such discs.

(4) *Request for certification; samples.* (i) In addition to complying with the requirements of § 431.1 of this chapter, a person who requests certification of a batch of antibiotic elution susceptibility discs shall submit with the request a statement showing the batch mark, the number of packages of each size in such batch, and the date on which the latest assay of the antibiotic used in making such batch was completed, the potency of each disc batch, the quantity of each ingredient used in making the batch, the date on which the latest assay of the drug constituting such batch was completed, and a statement that each ingredient used in

making the batch conforms to the requirements prescribed therefor by this section.

(ii) In connection with the request, such person shall submit results of the tests and assays made by him or her on an accurately representative sample of the batch for potency.

(iii) In connection with the request, such person shall submit an accurately representative sample of the batch, consisting of one disc for each 5,000 discs in the batch, but in no case collecting less than 100 discs. Single discs will be taken at regular intervals throughout the entire time of packaging the batch.

(b) *Tests and methods of assay for potency of antibiotic elution susceptibility discs*—(1) *Preparation for assay.* Use culture media as directed in § 460.6(a).

(2) *Test organisms*—(i) *Culture of test organism suspensions.* For each test organism listed in the following table, select the appropriate medium (as listed in § 460.6(a)), incubation period of the Roux bottle, and suggested storage period under refrigeration for the particular test organism.

Test organism	Medium used for			
	Method used	Slants	Roux bottles	Storage [2]
Suspension 1—*Staphylococcus aureus* (ATCC 29737) [1]	1	A	A	2 weeks.
Suspension 2—*Staphylococcus aureus* (ATCC 13150) [1]	1	A	A	1 week.
Suspension 3—*Pseudomonas aeruginosa* (ATCC 25619) [1]	6	J	J	1 week.
Suspension 4—*Klebsiella pneumoniae* (ATCC 10031) [1]	1	A	A	2 weeks.
Suspension 5—*Micrococcus luteus* (ATCC 9341) [1]	2	A	A	2 weeks.
Suspension 6—*Bordetella bronchiseptica* (ATCC 4617) [1]	1	A	A	2 weeks.
Suspension 7—*Streptococcus faecalis* (ATCC 14506) [1]	3	E	—	3 days.
Suspension 8—*Staphylococcus epidermidis* (ATCC 12228) [1]	4	A	—	3 days.
Suspension 9—*Bacillus subtilis* (ATCC 6633) [1]	5	A	B	1 year.

[1] Available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852.
[2] Storage period under refrigeration.

(ii) *Methods of preparation of test organism suspensions—(a) Method 1.* Maintain organisms on agar slants containing 10 milliliters of the appropriate medium. Transfer organisms to fresh slants using an inoculating loop. Streak the fresh slants thoroughly. Incubate the slants for 24 hours at 37° C. Remove resulting growth from the agar slant with sterile glass beads. Transfer the cells onto a large agar surface, such as a Roux bottle, containing 250 milliliters of the appropriate medium. Spread the cells over the entire surface of the Roux bottle. Incubate the Roux bottle for 24 hours at 37° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile U.S.P. saline test solution.

(b) Method 2. Proceed as directed in paragraph (b)(2)(ii)(*a*) of this section, except wash the growth from the surface of the Roux bottle with 20 milliliters sterile U.S.P. saline test solution.

(c) Method 3. Using an inoculation loop, transfer a portion of the growth on the slant to a culture tube containing 10 milliliters of sterile medium of the following composition:

Calf brains, infusion from, 200.0 gm.
Beef heart, infusion from, 250.0 gm.
Proteose peptone, 10.0 gm.
Dextrose, 2.0 gm.
Sodium cloride, 5.0 gm.
Sodium phosphate dibasic, 2.5 gm.
Distilled water, q.s. pH 7.4 after sterilization, 1,000.0 ml.
Incubate for 24 hours at 37° C.

(d) Method 4. Proceed as directed in paragraph (b)(2)(ii)(*c*) of this section, except transfer growth from the slant to a culture tube containing 50 milliliters of Medium D.

(e) Method 5. Proceed as directed in paragraph (b)(2)(ii)(*a*) of this section. Incubate the Roux bottle for 7 days at 37° C. Centrifuge the suspension at 3,500 RPM for 30 minutes. Decant the supernatant liquid. Resuspend the sediment in 50 milliliters sterile U.S.P. saline test solution. Heat-shock the suspension by placing in a 70° C. water bath for 30 minutes.

(f) Method 6. Proceed as directed in paragraph (b)(2)(ii)(*b*) of this section, except use 20 milliliters of Medium K.

(3)(i) *Preparation of plates.* Use volumes of appropriate media and plates as directed in § 460.6(c)(1) and (2).

(ii) *Inoculum and media to be used.* Depending on the particular antibiotic in the disc to be tested, select from the following table the inoculum and media to be used:

Antibiotic	Medium		Suspension No.	Volume [3]	Incub. temp.
	Base layer	Seed layer			
				ml	° *C*
Ampicillin (.22 mcg)	E	A	1	1.5	32–35
Ampicillin (4.5 mcg)	A	L	2	[1] 1.0	32–35
B acitracin	A	L	2	[1] 1.0	32–35
Carbenicillin	A	L	3	5.0	37
Cephalothin	A	L	4	1.0	37
Chloramphenicol	A	L	5	1.0	32–35
Clindamycin	C	L	1	2.0	32–35
Colistin	G	L	6	0.3	37
Doxycycline	E	A	5	0.1	32–35
Erythromycin	C	L	7	0.2	37
Gentamicin	C	L	2	0.1	32–35
Kanamycin	A	L	1	[1] 1.0	32–35
Methicillin	A	L	2	0.1	32–35
Neomycin	C	L	8	6.0	32–35
Novobiocin	C	L	9	[2] 0.2	37
Oleandomycin	A	L	2	[2] 1.0	32–35
Penicillin	E	A	1	1.5	32–35
Streptomycin	C	L	1	0.1	32–35
Tetracycline	E	A	5	0.1	32–35
Tobramycin	C	C	2	0.1	32–35
Vancomycin	C	L	3	2.0	32–35

[1] Prepare a 1:100 dilution of the bulk suspension in sterile U.S.P. saline test solution. Use the indicated quantity of the diluted suspension to inoculate the seed medium.
[2] Prepare a 1:10 dilution of the bulk suspension in sterile U.S.P. saline test solution. Use the indicated quantity of the diluted suspension to inoculate the seed medium.
[3] Suggested volume of suspension to be added to each 100 ml of seed agar.

(4) *Preparation of standard discs.* Depending on the concentration of antibiotic contained in the disc to be tested, prepare a stock solution for the standard disc by dissolving an accurately weighed quantity of the working standard in the solvent indicated to obtain an appropriate stock solution. Make further dilutions as required in the solvent indicated to obtain the following concentrations required on the standard discs:

Antibiotic	Solvent	Standard disc concentrations
Ampicillin (0.22 mcg.)	Water	0.1, 0.2, 0.4 mcg.
Ampicillin (4.5 mcg.)	do	1, 5, 15 mcg.
Bacitracin	do	5, 15, 30 units.
Carbenicillin	Methanol	24, 80, 240 mcg.
Cephalothin	50-percent methanol.	7.5, 20, 60 mcg.
Chloramphenicol	do	2, 4, 8 mcg.
Clindamycin	do	1, 2, 4 mcg.
Colistin	Water	5, 12.5, 25 mcg.
Doxycycline	Methanol	0.25, 1, 3 mcg.
Erythromycin	do	1.25, 5, 22.5 mcg.
Gentamicin	Water	5, 10, 20 mcg.
Kanamycin	do	3, 15, 45 mcg.
Methicillin	do	2.5, 5, 10 mcg.
Neomycin	do	3, 15, 45 mcg.
Novobiocin	Methanol	1.25, 2.5, 5 mcg.
Oleandomycin	Water [1]	1.25, 5, 22.5 mcg.
Penicillin	do	0.1, 0.2, 0.4 unit.
Streptomycin	do	6.25, 25, 100 mcg.
Tetracycline	Methanol	0.25, 1, 3 mcg.
Tobramycin	Water	5, 10, 20 mcg.
Vancomycin	do	3, 15, 45 mcg.

[1] If the chloroform adduct of oleandomycin is used as the standard, dissolve the weighing in absolute ethanol to a stock concentration of 10,000 micrograms per milliliter. Dilute this solution in water to achieve the working concentrations.

Use round, blank discs that conform to § 460.6(d). Place blank discs on aluminum or stanless steel wire mesh that is supported to allow circulation of air above and below the discs. Prepare the desired number of discs for each standard disc concentration by accurately adding 0.02-milliliter aliquots of the appropriate concentration of standard solution to each disc. Dry the discs in circulating air. Store standard discs under refrigeration in the presence of desiccant for a period not to exceed 2 weeks. Determine the stability of stored standard discs by assaying them

at daily and weekly interveral using freshly prepared standard disc for comparison.

(5) *Assay*—(i) *Individual discs.* On each of three plates prepared as directed in paragraph (b)(3) of this section, place standard disc and two or more discs from each batch to be tested. The standard disc and the sample discs are placed on the plates in a circular pattern with random arrangement, with no disc being closer than 24 millimeters (on centers) to any other disc. Discs are placed on the plates within as short a period of time as possible (not to exceed 3 minutes per plate) and tapped gently to ensure an even seal. After incubation as directed in paragraph (b)(3) of this section, measure the diameter of each circle of inhibition as accurately as possible. (In most cases, it is possible to estimate diameters to the nearest 0.1 millimeter).

(ii) *Estimation of potency.* Determine the logarithm of each dose of standard (x values) and the mean zone diameter for each dose of standard (y values). Using the three values of x and the three corresponding values of y, calculate Σx, Σx2, (Σx)2, Σy, and Σxy. Calculate the regression coefficient (slope, b) and the Y-intercept (a) of the standard response line by using the following equations:

$$b = \frac{n\Sigma xy - (\Sigma x)\ (\Sigma y)}{n\Sigma x^2 - (\Sigma x)^2}$$

$$a = \frac{\Sigma y - b\Sigma x}{n}$$

where n = the number of standard doses.

Determine the zone diameter (Y) for each sample disc being tested. Using the regression equation

$$X = \text{antilog}\,\frac{Y - a}{b}$$

calculate the concentration (X) for the mean response (Y) of the sample discs.

(6) *Potency.* The potency of the batch is satisfactory if the mean result obtained for the batch is not less than 85 percent and not more than 150 percent of that represented.

[45 FR 20668, Apr. 6, 1979]

§460.15 Streptomycin sulfate discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Streptomycin sulfate discs for use in culture media are paper discs intended for impregnation of culture media in the sensitivity testing of mycobacteria. They conform to all requirements and to all procedures prescribed by §460.1(a) for antibiotic sensitivity discs, except that each disc shall contain streptomycin sulfate equivalent to 10, 25, 50, or 500 micrograms of streptomycin.

(2) *Packaging.* It shall be packaged in accordance with the requirements of §460.1(b).

(3) *Labeling.* In addition to complying with the requirements of §460.1(c) of this chapter, the labeling shall also bear information indicating that the discs are for use in culture media for the sensitivity testing of mycobacteria and not for use in ordinary sensitivity disc plate tests.

(4) *Requests for certification; samples.* Requests for certification shall comply with §460.1(d).

(b) *Tests and methods of assay; potency.* Proceed as directed in §460.6 for the assay of streptomycin sulfate discs, except that:

(1) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 10, 25, or 50 micrograms of streptomycin, the control discs shall be made to contain the equivalent of 6.25, 12.5, 25, 50, and 100 micrograms of streptomycin per disc.

(2) In the assay of streptomycin sulfate discs labeled to contain the equivalent of 500 micrograms of streptomycin:

(i) To each 100 milliliters of seed agar used for the test add 2.0 milliliters of suspension number 11.

(ii) The control discs shall be made to contain the equivalent of 50, 100, 200, 400, and 800 micrograms of streptomycin per disc.

§460.16 Rifampin discs for use in culture media.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin discs for use in culture media are paper discs intended for impregnation of culture media in

the susceptibility testing of mycobacteria. They conform to all requirements and to all procedures prescribed by § 460.1(a) for antibiotic susceptibility discs, except that each disc shall contain 25 micrograms of rifampin activity.

(2) *Packaging.* It shall be packaged in accordance with the requirements of § 460.1(b).

(3) *Labeling.* In addition to complying with the requirements of § 460.1(c), the labeling shall also bear information indicating that the discs are for use in culture media for the susceptibility testing of mycobacteria and not for use in susceptibility tests of other microorganisms as described in § 460.1(c)(2).

(4) *Requests for certification; samples.* Requests for certification shall comply with § 460.1(d), except an accurately representative sample of the batch shall consist of one disc for each 5,000 in the batch, but in no case less than 100 discs collected by taking single discs at such intervals throughout the entire time of manufacturing the batch that the quantities manufactured during the intervals are approximately equal.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 460.6.

[41 FR 53476, Dec. 7, 1976]

Subpart B—Susceptibility Powders

§ 460.25 Bacitracin diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin diagnostic sensitivity powder is bacitracin, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to bacitracin. Each vial contains 2,000 units of bacitracin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The bacitracin used conforms to the standards prescribed by § 448.10a(a)(1) (i), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of units of bacitracin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The bacitracin used in making the batch for potency, moisture, and pH.

(*b*) The batch for potency sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The bacitracin used in making the batch: 6 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§460.28 Disodium carbenicillin diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Disodium carbenicillin diagnostic sensitivity powder is disodium carbenicillin packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to carbenicillin. Each vial contains disodium carbenicillin equivalent to not more than 1.0 gram of carbenicillin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 120 percent of its labeled content. It is sterile. Its moisture content is not more than 6 percent. When reconstituted as directed in the labeling, its pH is not less than 6.0 and not more than 8.0. The disodium carbenicillin used conforms to the standards prescribed by §440.13a(a)(1) (i), (v), (vi), and (vii) of this chapter.

(2) *Packaging.* It shall conform to the packaging requirements of §432.1 of this chapter.

(3) *Labeling.* In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of carbenicillin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The disodium carbenicillin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The disodium carbenicillin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 20 micrograms of carbenicillin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§460.33 Clindamycin hydrochloride hydrate sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin hydrochloride hydrate diagnostic sensitivity powder is clindamycin hydrochloride hydrate packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to clindamycin. Each vial contains clindamycin hydrochloride hydrate equivalent to 20 milligrams of clindamycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the amount of clindamycin it is represented to contain. It is sterile. Its moisture content is not more than 6.0 percent. Its pH in a solution containing

20 milligrams of clindamycin per milliliter is not less than 3.0 and not more than 6.5. The clindamycin hydrochloride hydrate used conforms to the standards prescribed by § 453.20(a)(1) (i), (ii), (iv), (v), (vi), and (vii) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnosis only.''

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of clindamycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Request for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The clindamycin hydrochloride hydrate used in making the batch for clindamycin content, microbiological activity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The clindamycin hydrochloride hydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when the vial is reconstituted with 1.0 milliliter of distilled water.

§ 460.38 Sodium colistimethate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium colistimethate diagnostic sensitivity powder is sodium colistimethate packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to sodium colistimethate. Each vial contains sodium colistimethate equivalent to 100 milligrams of colistin base activity. It is sterile. Its moisture content is not more than 9.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.5 and not more than 9.0. It gives a positive identity test for sodium colistimethate. The sodium colistimethate used conforms to the standards prescribed by § 448.20a(a)(1) (i), (vi), (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making serial dilutions for microbial susceptibility testing.

(3) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container.

(*a*) The statements ''Not for therapeutic use'' and ''For laboratory diagnosis only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of colistin base activity in each immediate container.

(*e*) The statements ''Stock solutions are stable for 14 days when refrigerated. For periods of storage up to 6 months, they should be frozen''.

(*f*) Its expiration date which is 12 months, except that the date may be used that is 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium colistimethate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, moisture, pH, and identity.

(ii) Samples required:

(*a*) The sodium colistimethate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 448.20a(b)(1) of this chapter, except prepare the sample for assay as follows: Reconstitute as directed in the labeling and further dilute with 10 percent potassium phosphate buffer, pH 6.0, to the proper prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of colistin base activity that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 440.80a(b)(5)(i) of this chapter.

(4) *pH.* Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using the drug reconstituted as directed in the labeling.

(5) *Identity.* Proceed as directed in § 448.20a(b)(7) of this chapter.

§ 460.42 Dihydrostreptomycin sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Dihydrostreptomycin sulfate sensitivity powder is crystalline dihydrostreptomycin sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to dihydrostreptomycin. Each vial contains dihydrostreptomycin sulfate equivalent to 20 milligrams of dihydrostreptomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.0. The dihydrostreptomycin sulfate used conforms to the standards prescribed by § 444.10a(a)(1) of this chapter, except the standards for sterility, pyrogens, and depressor substances. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of dihydrostreptomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The dihydrostreptomycin sulfate used in making the batch for potency, moisture, pH, streptomycin content, and crystallinity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The dihydrostreptomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 19181, May 30, 1974, as amended at 46 FR 60569, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

§ 460.47 Doxycycline hyclate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate diagnostic sensitivity powder is crystalline doxycycline hyclate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to doxycycline. Each vial contains doxycycline hyclate equivalent to 20 milligrams of doxycycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 4 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The doxycycline hyclate used conforms to the standards prescribed by § 446.20(a)(1) (i), (iii), (iv), (v), and (vi) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of doxycycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility moisture, and pH.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.1 microgram of doxycycline per milliliter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§460.55 Lincomycin hydrochloride monohydrate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Lincomycin hydrochloride monohydrate diagnostic sensitivity powder is lincomycin hydrochloride monohydrate powder packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to lincomycin. Each vial contains 20 milligrams of lincomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of lincomycin that it is represented to contain. It is sterile. Its moisture content is not more than 7 percent. It gives a positive identity test for lincomycin hydrochloride monohydrate. The lincomycin hydrochloride monohydrate used conforms to the standards prescribed by §453.30(a)(1) (i), (iii), (iv), (v), and (ix) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile broth medium when preparing a stock solution for use in making serial dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statements ''Not for therapeutic use'' and ''For laboratory diagnosis only''.

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of lincomycin in each immediate container.

(*e*) The statements ''Store in a refrigerator'' and ''Reconstituted solutions should be refrigerated.''

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch for potency, moisture, pH, crystallinity, and specific rotation.

(*b*) The batch for potency, sterility, moisture, and identity.

(ii) Samples required:

(*a*) The lincomycin hydrochloride monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 2.0 micrograms of lincomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Identity.* In a 100-milliliter beaker, dissolve sufficient sample to yield a concentration of at least 80 milligrams per milliliter, using no more than 2.0 milliliters of water. Add acetone until precipitation begins and then add an additional 20 milliliters of acetone. Filter the solution through filter paper and wash with two 10-milliliter portions of acetone. Expose the residue at room temperature until it is dry enough to be reduced to moderately fine particles. Dry the material for 4 hours in a 60° C. vacuum oven. After drying the material, care must be taken to avoid extended exposure to the atmosphere. The infrared spectrum of a mineral oil dispersion of the residue thus obtained exhibits maxima at the same wavelengths as that of the lincomycin working standards, similarly treated.

§ 460.58 Methacycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride diagnostic sensitivity powder is the crystalline methacycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to methacycline. Each vial contains methacycline hydrochloride equivalent to 20 milligrams of methacycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1) (i), (iii), (v), and (vi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile.''

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample as follows: Reconstitute as directed in the labeling. Dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.06 microgram of methacycline per milliliter.

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture*. Proceed as directed in §436.201 of this chapter.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§460.64 Minocycline hydrochloride powder for microbial susceptibility testing.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Minocycline hydrochloride powder for microbial susceptibility testing is minocycline hydrochloride with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the susceptibility of microorganisms to minocycline. Each vial contains minocycline hydrochloride equivalent to 20 milligrams of minocycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 4.0. The minocycline hydrochloride used conforms to the standards prescribed by §446.60(a)(1) (i), (iii), (iv), (v), (vi), and (vii) of this chapter.

(2) *Packaging*. The immediate container shall be of colorless, transparent glass, and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling*. In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of minocycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples*. In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, minocycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for

assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 0.100 microgram of minocycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.66 Oleandomycin phosphate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oleandomycin phosphate diagnostic sensitivity powder is oleandomycin phosphate, with or without one or more suitable buffers and dilutents, packaged in vials and intended for use in clinical laboratories, for determining in vitro the sensitivity of microorganisms to oleandomycin. Each vial contains oleandomycin phosphate equivalent to 20 milligrams of oleandomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.0 and not more than 7.0. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of oleandomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oleandomycin phosphate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The oleandomycin phosphate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.70 Oxytetracycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Oxytetracycline hydrochloride diagnostic sensitivity powder is crystalline oxytetracycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to oxytetracycline. Each vial contains oxytetracycline hydrochloride equivalent to 20 milligrams of oxytetracycline. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 2.0 and not more than 3.5. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1) (i), (vi), (vii), (viii), and (ix) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of oxytetracycline in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.75 Potassium penicillin G diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Potassium penicillin G diagnostic sensitivity powder is crystalline potassium penicillin G, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to penicillin G. Each vial contains 20,000 units of penicillin G. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 1.5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The potassium penicillin G used conforms to the standards prescribed by § 440.80a(a)(1) (i), (v), and (vi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of units of penicillin G in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The penicillin G used in making the batch for potency, moisture, pH, and crystallinity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The potassium penicillin G used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e) (1) or (2) of that section, except if using the method in paragraph (e)(2), use medium B in lieu of medium A.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.79 Polymyxin B sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate diagnostic sensitivity powder is polymyxin B sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to polymyxin B. Each vial contains the equivalent of 20,000 units of polymyxin B. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 7.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.5. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30a(a)(1) (i), (v), (vi), and (ix) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of units of polymyxin B in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.86 Spectinomycin hydrochloride powder for microbial susceptibility testing.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Spectinomycin hydrochloride powder for microbial susceptibility testing is spectinomycin dihydrochloride pentahydrate with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the susceptibility of microorganisms to spectinomycin. Each vial contains spectinomycin hydrochloride equivalent to 100 milligrams of spectinomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its moisture content is not more than 8 percent. When reconstituted as directed in the labeling, its pH is not less than 3.8 nor more than 5.6. The spectinomycin hydrochloride used conforms to the standards prescribed by § 455.80a(a)(1) (i), (ii), (vii), (viii), (ix), (x), and (xi) of this chapter.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of the amount of sterile diluent prescribed in the labeling when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement, ''For laboratory use only''.

(*b*) The statement, ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of spectinomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The spectinomycin hydrochloride used in making the batch for spectinomycin content, microbiological activity, moisture, pH, identity, residue on ignition, and crystallinity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The spectinomycin hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§ 460.89 Streptomycin sulfate diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Streptomycin sulfate diagnostic sensitivity powder is streptomycin sulfate, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to streptomycin. Each vial contains streptomycin sulfate equivalent to 20 milligrams of streptomycin. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 7.0. The streptomycin sulfate used conforms to the standards prescribed by § 444.70a(a)(1) (i), (vi), and (vii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging*. The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling*. In addition to the requirements of § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only''.

(*b*) The statement ''Sterile''.

(*c*) The batch mark.

(*d*) The number of milligrams of streptomycin in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The streptomycin sulfate used in making the batch for potency, loss on drying, and pH.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The streptomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with sterile distilled water to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling.

§460.93 Tetracycline hydrochloride diagnostic sensitivity powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride diagnostic sensitivity powder is crystalline tetracycline hydrochloride, with or without one or more suitable buffers and diluents, packaged in vials and intended for use in clinical laboratories for determining in vitro the sensitivity of microorganisms to tetracycline. Each vial contains 20 milligrams of tetracycline hydrochloride. The potency of each immediate container is satisfactory if it contains not less than 90 percent and not more than 115 percent of its labeled content. It is sterile. Its loss on drying is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.8 and not more than 3.0. The tetracycline hydrochloride used conforms to the standards prescribed by §446.81a(a)(1) (i), (vi), and (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* The immediate container shall be of colorless, transparent glass and it shall be a tight container as defined by the U.S.P. It shall be so sealed that the contents cannot be used without destroying such seal. It shall be of appropriate size to permit the addition of 20 milliliters of sterile diluent when preparing a stock solution for use in making further dilutions for microbial susceptibility testing.

(3) *Labeling.* In addition to the requirements of §432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On its outside wrapper or container and on the immediate container:

(*a*) The statement ''For laboratory diagnostic use only.''

(*b*) The statement ''Sterile.''

(*c*) The batch mark.

(*d*) The number of milligrams of tetracycline hydrochloride in each immediate container.

(ii) On the circular or other labeling within or attached to the package, adequate information for use of the drug in the clinical laboratory.

(4) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, moisture, pH, crystallinity, and absorptivity.

(*b*) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the prescribed reference concentration.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter using the drug reconstituted as directed in the labeling.

Subpart C—Susceptibility Test Panels

SOURCE: 43 FR 9793, Mar. 10, 1978, unless otherwise noted.

§ 460.100 Antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Antimicrobial susceptibility test panels are polystyrene trays molded with separate wells which contain frozen aliquots of antibiotic and non-antibiotic antimicrobial solutions in Mueller-Hinton broth. The trays are used in clinical laboratories for determining susceptibility of microorganisms to antimicrobial drugs. The broth antimicrobial solutions are prepared from serially diluted antimicrobial stock solutions. The concentrated aqueous antimicrobial stock solutions must conform to the requirements for certification prescribed by this section.

(2) *Labeling.* In addition to the requirements of §§ 432.5 and 809.10 of this chapter, each test panel shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or immediate container of trays:

(*a*) The batch mark.

(*b*) The name and potency of each solution in the batch according to the following:

Name of drug	Content of antimicrobic in micrograms per milliliter
Ampicillin (gram-positive panel)	8, 4, 2, 1, 0.5, 0.25, 0.12.
Ampicillin (gram-negative panel)	16, 8, 4, 2, 1, 0.5, 0.25.
Carbenicillin	512, 256, 128, 64, 32, 16, 8.
Cephalothin	64, 32, 16, 8, 4, 2, 1.
Chloramphenicol	32, 16, 8, 4, 2, 1, 0.5.
Clindamycin	16, 8, 4, 2, 1, 0.5, 0.25.
Colistin	4.
Erythromycin	16, 8, 4, 2, 1, 0.5, 0.25.
Gentamicin	16, 8, 4, 2, 1, 0.5, 0.25.
Kanamycin	64, 32, 16, 8, 4, 2, 1.
Methicillin	16, 8, 4, 2, 1, 0.5, 0.25.
Penicillin G	4, 2, 1, 0.5, 0.25, 0.12, 0.06.
Tetracycline	16, 8, 4, 2, 1, 0.5, 0.25.
Tobramycin	16, 8, 4, 2, 1, 0.5, 0.25.
Nitrofurantoin	64.
Trimethoprim (Gram-positive and gram-negative panel).	32, 16, 8, 4, 2, 1, 0.5.
Trimethoprim (Combination identification panel).	2.
Sulfamethoxazole (Gram-positive and gram-negative panel).	608, 304, 152, 76, 38, 19, 9.5.
Sulfamethoxazole (Combination identification panel).	38.

(*c*) The statement ''For in vitro diagnostic use''.

(ii) On each tray: The name of the panel, the expiration date, and the batch mark, including filling operation identification.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The concentrated antimicrobial stock solutions used in making the batch for potency and pH.

(*b*) The antimicrobial susceptibility test panels from the batch for performance and identity.

(ii) Samples required: (*a*) The concentrated antimicrobial stock solutions used in making the batch as directed in each individual monograph.

(*b*) The batch: A minimum of 25 panels selected at such intervals throughout the entire time of the filling operation so that the quantities of panels filled during the intervals are approximately equal.

(b) *Tests and methods of assay*—(1) *Performance*—(i) *Procedure.* Test randomly selected panels with each of the four test organisms as follows: Use the test organism suspensions prepared as described in § 460.6(b) (14), (15), (16), and (17). Transfer 0.005 milliliter of the appropriate test organism suspension into all wells of a panel. For the purpose of this section, wells are identified 1–10 from left to right and A–H from front to rear. Incubate inoculated panels in groups of three or less, each with a clean cover, at 35° C for 16 to 18 hours. For each control organism, the lowest concentration showing complete inhibition of growth is the minimal inhibitory concentration for that particular antimicrobial agent (referred to hereafter as its end point). For the purpose of this section, an on-scale end point means an end point which has been established by growth in the next lower concentration well. No growth in any well in row G does not indicate an on-scale end point unless the next lower concentration can be shown to produce growth. Establishment of an end point for a no-growth result in a well in row G requires additional testing in which 0.1 milliliter of sterile medium N is added to well G in some panels prior to inoculation with the test organism. An on-scale end point in row

G is established by no growth in the undiluted well G and growth in the two-fold diluted well G.

(ii) *Evaluation.* Susceptibility test panels of each filling operation pass the performance test if the on-scale end point for each antimicrobial agent and each test organism meet the limits specified in the following table (allowable off-scale end points are identified by asterisks):

PERFORMANCE END POINT ACCEPTANCE LIMITS

Antimicrobic	END POINTS (MICROGRAMS PER MILLILITER)			
	E. coli (ATCC 25922)	*S. faecalis* (ATCC 29212)	*S. aureus* (ATCC 29213)	*P. aeruginosa* (ATCC 27853)
Ampicillin	1–4	0.5–2	0.25–1	Greater than 8 (gram-positive panel). Greater than 16 (gram-negative panel).
Carbenicillin	4*–16	16–64	No growth	16–64.
Cephalothin	4–16	16–64	.....do	64-greater than 64.
Chloramphenicol	2–8	4–16	4–16	Greater than 32.
Clindamycin	16-greater than 16.	4–16	No growth	Greater than 16.
Colistin	No growth	Growth	Growth	No growth.
Erythromycin	16-greater than 16.	1–4	0.125*–0.5	Greater than 16.
Gentamicin	0.125*–0.5	4–16	0.125*–0.5	0.125*–0.5
Kanamycin	1–4	16–64	0.5*–2	Greater than 64.
Methicillin	Greater than 16.	Greater than 16.	0.5–2	Greater than 16.
Nitrofurantoin	No growth	No growth	No growth	Growth.
Penicillin G	Greater than 4.	1–4	0.125–0.5	Greater than 4.
Tetracycline	0.25–1	8-greater than 16.	0.125*–0.5	4–16.
Trimethoprim-sulfamethoxazole.	No growth	No growth	No growth	16/304-greater than 32/608 (gram-positive and gram-negative panel).
	.....do	.....do	.....do	Growth (combination identification panel).
Tobramycin	0.25–1	8-greater than 16.	0.125*–0.5	No growth.

*An additional two-fold dilution below well G.

(2) *Identity*—(i) *Test procedure.* Test a randomly selected panel. Use the test organism suspensions prepared as described in § 460.6(b) (18), (19), and (20), and use one panel for several organisms as shown in the following table:

Test organism			Identity test data			
Name	ATCC No.	Suspension No.	Antibiotics tested	Type of panel and well to be inoculated		Response
				Gram-positive	Gram-negative	
S. aureus	29247	18	Methicillin	3A	.....	No growth.
			Penicillin	4A	.....	Growth.
			Erythromycin	2E	.....	Do.
			Cephalothin	6D	2D	No growth.
			Clindamycin	1E	4D	Do.
			Tetracycline	8D	.....	Growth.
Ent. cloacae	29249	19	Cephalothin	.....	2B	Do.
			Kanamycin	.....	7B	No growth.
Ps. aeruginosa	29248	20	Gentamicin	.....	3D	Growth.
			Tobramycin	.....	8D	No growth.

(Rows are numbered 1–10 from left to right; A–H from front to rear.)

Transfer 0.005 milliliter of the test organism suspension into designated wells of the panel. Incubate the panels at 35° C for 16 to 18 hours in covered stacks of three or fewer panels. Read the designated wells for growth or no growth.

(ii) *Evaluation.* Each susceptibility test panel passes the identity test if the determinations of growth in the designated wells agree with those shown in the table in paragraph (b)(2)(i) of this section.

[43 FR 9793, Mar. 10, 1978; 43 FR 12859, Mar. 28, 1978]

§ 460.110 Ampicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Ampicillin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous ampicillin trihydrate stock solutions serially diluted with distilled water to contain approximately 3,200, 1,600, 800, 400, 200, 100, and 50 micrograms ampicillin per milliliter. The potency of each solution is satisfactory if it is not less than 100 percent and not more than 150 percent of the number of micrograms of ampicillin that it is represented to contain. The pH of the solution containing 3,200 micrograms of ampicillin per milliliter is not less than 4.0 and not more than 7.0. The ampicillin trihydrate used conforms to the requirements of § 440.7(a)(1) (i), (iii), (iv), (v), (vi), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 2.5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 3,200 micrograms of ampicillin per milliliter.

§ 460.113 Carbenicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Carbenicillin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous carbenicillin disodium stock solutions serially diluted with distilled water to contain approximately the following concentrations: 20,480, 10,240, 5,120, 2,560, 1,280, 640, and 320 micrograms of carbenicillin per milliliter. The potency of each solution is satisfactory if it is not less than 100 percent and not more than 150 percent of the number of micrograms of carbenicillin that it is represented to contain. The pH of the solution containing 20,480 micrograms of carbenicillin per milliliter is not less than 6.0 and not more than 8.0. The carbenicillin disodium used conforms to the requirements of § 440.13a (a)(1) (i), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 20 micrograms of carbenicillin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 20,480 micrograms of carbenicillin per milliliter.

§ 460.116 Cephalothin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cephalothin concentrated stock solutions for use in preparing susceptibility test panels are frozen cephalothin sodium aqueous stock solutions serially diluted with distilled water to contain approximately the following concentrations: 2,560, 1,280, 640, 320, 160, 80, and 40 micrograms of cephalothin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of cephalothin that it is represented to contain. The pH of the solution containing 2,560 micrograms of cephalothin per milliliter is not less than 4.2 and not more than 7.0. The cephalothin used conforms to the standards prescribed by § 442.25a(a)(1) (i), (v), (vi), (vii), (viii), and (ix) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions used for testing must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 microgram of cephalothin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 2,560 micrograms of cephalothin per milliliter.

§ 460.119 Chloramphenicol concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol concentrated stock solutions for use in preparing susceptibility test panels are frozen aqueous chloramphenicol stock solutions serially diluted with distilled water to contain approximately the following concentrations: 1,280, 640, 320, 160, 80, 40, and 20 micrograms of chloramphenicol per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of chloramphenicol that it is represented to contain. The pH of the solution containing 1,280 micrograms of chloramphenicol per milliliter is not less than 4.5 and not more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1) (i), (iii), (iv), (v), (vi), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solution must be thawed and brought to room temperature before further testing.

(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter using the solution containing 1,280 micrograms of chloramphenicol per milliliter.

§ 460.122 Clindamycin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clindamycin concentrated stock solutions for use in preparing susceptibility test panels are frozen aqueous clindamycin hydrochloride stock solutions serially diluted with distilled water to contain approximately the following concentrations: 640, 320, 160, 80, 40, 20, and 10 micrograms of clindamycin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of clindamycin that it is represented to contain. The pH of the solution containing 640 micrograms of clindamycin per milliliter is not less than 4.5 and not more than 7.0. The clindamycin used conforms to the standards prescribed by § 453.20(a)(1) (i), (ii), (iv), (v), (vi) and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter. Prepare the sample for assay by diluting an accurately measured representative portion of the sample with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of clindamycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 640 micrograms of clindamycin per milliliter.

§ 460.125 Colistin concentrated stock solution for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous colistin sulfate stock solutions serially diluted with distilled water to contain an approximate concentration of 160 micrograms of colistin per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of colistin that it is represented to contain. Its pH is not less than 5.0 and not more than 8.0. The colistin used conforms to the requirements of § 448.21(a)(1) (i), (iii), (iv), and (v) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: Five frozen aliquots of the concentrated stock solution containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solution must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 10 percent potassium phosphate buffer (solution 6), to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution without further dilution.

§ 460.128 Erythromycin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous erythromycin stock

solutions serially diluted with 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3) to contain approximately the following concentrations: 3,200, 1,600, 800, 400, 200, 100, and 50 micrograms of erythromycin per milliliter. The potency of each solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of erythromycin that it is represented to contain. The pH of the solution containing 3,200 micrograms of erythromycin per milliliter is not less than 7.5 and not more than 10.0. The erythromycin used conforms to the requirements of § 452.10(a)(1) (i), (iii), (iv), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter. Prepare the sample for assay by diluting an accurately measured representative portion of the sample with 0.1 *M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 1.0 microgram of erythromycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 3,200 micrograms of erythromycin per milliliter.

§ 460.131 Gentamicin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin concentrated stock solutions for use in preparing susceptibility test panels are frozen aqueous gentamicin sulfate stock solutions serially diluted with distilled water to contain approximately the following concentrations: 640, 320, 160, 80, 40, 20, and 10 micrograms of gentamicin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of gentamicin that it is represented to contain. The pH of the solution containing 640 micrograms of gentamicin per milliliter is not less than 4.5 and not more than 7.0. The gentamicin used conforms to the standards prescribed by § 444.20(a)(1) (iii), (iv), (v), and (vii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain.

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 640 micrograms of gentamicin per milliliter.

§ 460.134 Kanamycin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin concentrated stock solutions for use in preparing susceptibility test panels are frozen aqueous kanamycin sulfate stock solutions serially diluted with distilled water to contain approximately the following concentrations: 2,560, 1,280, 640, 320, 160, 80, and 40 micrograms of

kanamycin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of kanamycin that it is represented to contain. The pH of the solution containing 2,560 micrograms of kanamycin per milliliter is not less than 6.5 and not more than 8.5. The kanamycin used conforms to the standards prescribed by § 444.30(a)(1) (i), (iii), (iv), (vi), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Performance.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion with distilled water to the reference concentration of 10 micrograms of kanamycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 2,560 micrograms of kanamycin per milliliter.

§ 460.137 Methicillin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methicillin concentrated stock solutions for use in preparing susceptibility test panels are frozen aqueous methicillin sodium stock solutions serially diluted with distilled water to contain approximately 6,400, 3,200, 1,600, 800, 400, 200, and 100 micrograms of methicillin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 100 percent and not more than 150 percent for the number of micrograms of methicillin that it is represented to contain. The pH of the solution containing 6,400 micrograms of methicillin per milliliter is not less than 5.0 and not more than 7.5. The methicillin used conforms to the standards prescribed by § 440.36a(a)(1) (i), (v), (vi), (vii), (viii), and (ix) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Request for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 2.5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 10 micrograms of methicillin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 6,400 micrograms of methicillin per milliliter.

§ 460.140 Penicillin G concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Penicillin G concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous penicillin G potassium solutions serially diluted with distilled water to contain approximately 1,600, 800, 400, 200, 100, 50, and 25 micrograms of penicillin G per milliliter. The potency of each diluted solution is satisfactory if it is not less than 100 percent and not more than 150 percent of the number of micrograms of penicillin G that it is represented to

contain. The pH of the solution containing 1,600 micrograms of penicillin G per milliliter is not less than 5.0 and not more than 7.5. The penicillin G potassium used conforms to the standards prescribed by § 440.80a(a)(1) (i), (v) and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 2.5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 1.0 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 1.0 unit (0.600 microgram) of penicillin G per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 1,600 micrograms of penicillin G per milliliter.

§ 460.146 Tetracycline concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous tetracycline hydrochloride stock solutions serially diluted with distilled water to contain approximately the following concentrations: 640, 320, 160, 80, 40, 20, and 10 micrograms of tetracycline per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of tetracycline that it is represented to contain. The pH of the solution containing 640 micrograms of tetracycline per milliliter is not less than 3.0 and not more than 7.0. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1) (i), (vi), (vii), and (viii) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to the reference concentration of 0.24 microgram of tetracycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 640 micrograms of tetracycline per milliliter.

§ 460.149 Tobramycin concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin concentrated stock solutions for use in preparing antimicrobial susceptibility test panels are frozen aqueous tobramycin sulfate stock solutions serially diluted with distilled water to contain approximately the following concentrations: 1,280, 640, 220, 160, 80, 40, and 20 micrograms of tobramycin per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of tobramycin that it is represented to contain. The pH of the solution containing 1,280 micrograms of tobramycin per milliliter is not less than 8.5 and not more than 10.5. The tobramycin

used conforms to the standards prescribed by § 444.80(a)(1)(i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing approximately 5.0 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 1,280 micrograms of tobramycin per milliliter.

§ 460.152 Trimethoprim concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Trimethoprim concentrated stock solutions for use in antimicrobial susceptibility test panels are frozen aqueous acidified trimethoprim lactate stock solutions serially diluted with distilled water to contain approximately the following concentrations: 6,400, 3,200, 1,600, 800, 400, 200, and 100 micrograms of trimethoprim per milliliter, or to a single concentration of 400 micrograms of trimethoprim per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of trimethoprim that it is represented to contain. The pH of the solution, containing 400 micrograms of trimethoprim per milliliter, is not less than 2.5 and not more than 6.0. The trimethoprim lactate used is a white, odorless, crystalline powder. Its potency is not less than 74 percent nor more than 78 percent trimethoprim. Its melting range is between 183° C and 187° C. Its loss on drying is not more than 1.0 percent. It passes the identity test. It conforms to the standards prescribed by this section.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The batch for potency and pH.

(*b*) The trimethoprim lactate used in preparing the solutions for potency, melting range, loss on drying, and identity.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay*—(1) *Trimethoprim stock solution.* The sample solutions must be thawed and brought to room temperature before testing.

(i) *Potency*—(*a*) *Working standard.* Accurately weigh approximately 52 milligrams of the trimethoprim working standard. Dissolve and dilute the working standard in 100 milliliters of 0.1*N* hydrochloric acid to make a stock solution, containing approximately 400 micrograms of trimethoprim per milliliter. Further dilute the working standard twentyfold in distilled water to approximately 20 micrograms of trimethoprim per milliliter.

(*b*) *Preparation of sample.* The sample solution must be thawed and brought to room temperature. Further dilute with distilled water to an estimated concentration of 20 micrograms of trimethoprim per milliliter.

(*c*) *Procedure.* Using a suitable spectrophotometer equipped with 1.0 centimeter cells, determine the absorbance of the sample and working standard solutions at a wavelength of 270 nanometers.

(*d*) *Calculations.* Calculate the potency of the trimethoprim solutions as follows:

Micrograms of trimethoprim per milliliter in trimethoprim lactate=sample absorbance

times weight of standard (mg) times 0.763 times *f* times 1,000 times purity of standard in percent, divided by standard absorbance times 100 times 20 times 100

where:

f=dilution factor of each sample solution.

(ii) *pH.* [Reserved]

(2) *Trimethoprim*—(i) *Potency.* Proceed as directed in § 436.213 of this chapter using the method described in paragraph (e)(2) of that section except, to prepare the sample for assay, dissolve approximately 50 milligrams of sample accurately weighed in 60 milliliters of glacial acetic acid. Calculate the trimethoprim content as follows:

Percent trimethoprim=(V_1-V_2) (N) (290.3) (100) (100) divided by $(100-M)$ times W

where:

V_1=Volume perchloric acid used to titrate sample;
V_2=Volume perchloric acid used to titrate blank;
W=Sample weight in milligrams;
N=Normality of perchloric acid reagent;
M=Percent moisture in the sample.

(ii) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(iii) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(iv) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.25 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[43 FR 9793, Mar. 10, 1978; 43 FR 12859, Mar. 28, 1978]

§ 460.153 Sulfamethoxazole concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sulfamethoxazole concentrated stock solutions for use in antimicrobial susceptibility test panels are frozen aqueous alkaline sulfamethoxazole stock solutions serially diluted with distilled water containing approximately the following concentrations: 12,160, 6,080, 3,040, 1,520, 760, 380, and 190 micrograms of sulfamethoxazole per milliliter, or to a single concentration of 760 micrograms of sulfamethoxazole per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of sulfamethoxazole that it is represented to contain. The pH of the solution containing 760 micrograms of sulfamethoxazole per milliliter is not less than 9.0 and not more than 12.5. The sulfamethoxazole used conforms to the standards prescribed by the National Formulary.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 10 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Dilute aliquots of each sample in sufficient distilled water to make solutions containing 10 micrograms of sulfamethoxazole per milliliter. Place approximately 100 milligrams of the standard, accurately weighed, into a 100-milliliter volumetric flask and make to volume with 0.1*N* sodium hydroxide. Pipet 1.0 milliliter of this solution into a 100-milliliter volumetric flask and make to volume with distilled water. Using a suitable spectrophotometer equipped with 1.0 centimeter cells, and distilled water as the blank, determine the absorbance of sample and standard solutions at 257 nanometers. Calculate the potency of the sulfamethoxazole as follows:

Micrograms of sulfamethoxazole per milliliter=sample absorbance times weight of standard (in mcg) times *f* times purity of standard in percent divided by standard absorbance times 100 times 100

where:

f=Dilution factor of each sample solution.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 760 micrograms of sulfamethoxazole per milliliter.

[43 FR 9793, Mar. 10, 1978; 43 FR 12859, Mar. 28, 1978]

PARTS 461–499 [RESERVED]