

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 acetate 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: Sec. 507 of the Federal Food, Drug, and Cosmetic Act (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)] - N¹ - [(s) - 4 - amino - 2 - hydroxy - 1 - oxobutyl] - 2 - deoxy - D - streptomine. 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*-streptomine, *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 (H₂SO₄) 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 H₂SO₄ 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 H₂SO₄ is 1:2 and not less than 6.0 and not more than 7.3 if the molar ratio of amikacin to H₂SO₄ 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 0.02M potassium dihydrogen phosphate and methanol, high performance liquid chromatography reagent grade (28:72 by volume). Adjust the pH to 6.5 with 0.4M 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_t) 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) *Coefficient 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:

$$\text{Micrograms of amikacin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

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 amikacin 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 50 milliliters 0.1N HCl.

(b) 0.25 percent ferric chloride solution. Dilute 2.5 milliliters of 10 percent ferric chloride in 0.1N HCl to 100 milliliters with 0.01N 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 1N 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 1.2N HCl. To each flask add 5.0 milliliters of 0.25 percent ferric chloride reagent, make to vol-

ume with distilled water, and mix thoroughly. Transfer the colored solutions to 2.0-centimeter absorption cells and measure the percent light transmission at 530 m μ 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 = $(\text{Milligrams of streptomycin} \times 100) / (\text{Milligrams of dihydrostreptomycin found in the sample used})$

(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 $+107^\circ$ and not more than $+121^\circ$.

(vi) Its content of gentamicin C₁ 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 C₂, 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 C₁, C_{1a}, and C₂, 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 0.1M 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 C₁, C₁*

(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 de-

scribed 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 C₁ 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 C₂, 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 C₁, C_{1a}, and C₂, 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 0.1M 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 C₁, C_{1a}, and C₂*—(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 glacial 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 C₁, the one closest is gentamicin C_{1a}, and the middle zone is gentamicin C₂. Cut the corresponding zones out of the other unsprayed half of the sheet. Cut each portion of the sheet thus obtained into small strips and put those from each zone into a separate 125-milliliter glass-stoppered flask. Add 50 milliliters of 0.1M 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 0.1M 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 6N hydrochloric acid, and tightly close the container. Heat in a water bath at 100° C. for 1 hour and cool. Add 4 milliliters of 6N sodium hydroxide, then dilute with sterile 0.1M 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 0.1M 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:

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

$$L = \frac{3a + 2b + c - e}{5}$$

$$H = \frac{3e + 2d + c - a}{5}$$

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 1.0-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 0.1M 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 epidermidis (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.:

(I) 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)(I) 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 0.1M 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 0.1M 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)(I) of this section. Using 3 milliliters of U.S.P. saline

¹Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

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 m μ 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 \times 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 (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 0.1M 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 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

¹Available from: American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852.

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-Streptomine, 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 solu-

tion 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 0.1M 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 0.1M 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 of 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 repackaging 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 50 milliliters of 0.1N HCl.

(b) 0.25 percent ferric chloride solution: Dilute 2.5 milliliters of 10 percent ferric chloride in 0.1N HCl to 100 milliliters with 0.01N 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 1N 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 \rightarrow 4)-*0*-[2,6-diamino-2,3,6-trideoxy- α -*D*-ribohexopyranosyl-(1 \rightarrow 6)]-2-deoxy-L-streptomine. 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 re-

quirements 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 =
$$\frac{[(A - B) \times (\text{normality of perchloric acid reagent}) \times 93.4 \times 100 \times 1,000]}{(\text{Weight of sample in milligrams} \times (100 - m))}$$

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 \rightarrow 4)-*0*-[2,6-diamino-2,3,6-trideoxy- α -*D*-ribohexopyranosyl-(1 \rightarrow 6)]-2-deoxy-L-streptomine. 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 repackaging 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 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 0.1M potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute in 0.1M 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 0.1M 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 0.1M 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 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 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 0.1M 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 0.1M 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 0.1M 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 con-

tainer. 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 0.1M 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 0.1M 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) Gentamicin 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 0.1M 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 alcohol, 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 0.1M 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 \pm 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 milligrams \pm 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 \pm 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.

(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 0.1M 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 hydrocortisone, 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 0.1M 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 acetone 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 acetone 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 0.1M potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Make proper estimated dilutions to the prescribed reference concentration with 0.1M 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 0.1M 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 0.1M 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 re-

quirements 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 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.

[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_a = 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. Degass 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 methanol: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_p) 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:

$$\begin{array}{l} \text{Milligrams of} \\ \text{fluorometholone} \\ \text{acetate} \\ \text{per container} \end{array} = \frac{A_U \times P_s \times d}{A_s \times 1,000}$$

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 0.1M 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 0.1M 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 0.1M 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 C₁, C_{1a}, and C₂, 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 C₁, C_{1a}, and C₂, 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 0.1M 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 acetate; 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 methylprednisolone; or

(e) 1.0 milligram of triamcinolone acetonide; or

(f) 2.5 milligrams or 5.0 milligrams of prednisolone acetate; or

(g) 15.0 milligrams 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 0.1M 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 0.1M 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 0.1M potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 0.1M 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 pre-

scribed 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: Transfer an accurately weighed representative portion into a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1M potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration and blend 3 to 5 minutes. Further dilute with 0.1M 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.

- (i) 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 0.1M potassium phosphate buffer, pH 8, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make further dilutions with 0.1M 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 acetate 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 acetate ointment is an ointment containing, in each gram, 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetate. 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, resi-

due 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 than 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 acetone cream.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin-triamcinolone acetone cream is a cream containing 2.5 milligrams of neomycin, 0.25 milligram of gramicidin, and 1.0 milligram of triamcinolone acetone 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 acetone 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 acetone 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 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 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 0.1M 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 Demeclocycline 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.