

(a) The nystatin used in making the batch: 10 immediate containers of approximately 300 milligrams each.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets for 3 to 5 minutes in a high-speed glass blender with sufficient dimethylformamide to give a convenient concentration. Dilute an aliquot with sufficient dimethylformamide to give a stock solution containing 400 units of nystatin per milliliter (estimated). Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Loss on drying*. Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time*. Proceed as directed in § 436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section, except use distilled water in lieu of gastric fluid.

[39 FR 19134, May 30, 1974. Redesignated at 43 FR 43458, Sept. 26, 1978]

§ 449.650b Nystatin vaginal suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Nystatin vaginal suppositories contain in each suppository 100,000 units of nystatin in a suitable and harmless water soluble base. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of nystatin that it is represented to contain. Its moisture content is not more than 1.5 percent. The nystatin used conforms to the standards prescribed by § 449.50(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The nystatin used in making the batch for potency, loss on drying, pH, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The nystatin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch: A minimum of 30 suppositories.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of suppositories into a high-speed glass blender jar containing sufficient dimethylformamide to give a convenient concentration. Blend for 3 to 5 minutes. Dilute an aliquot with sufficient dimethylformamide to obtain a concentration of 400 units of nystatin per milliliter (estimated). Further dilute an aliquot with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 20 units of nystatin per milliliter (estimated).

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

[43 FR 43458, Sept. 26, 1978, as amended at 50 FR 19920, May 13, 1985]

PART 450—ANTITUMOR ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.

- 450.10a Sterile bleomycin sulfate.
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- 450.240 Plicamycin for injection.
- 450.245 Mitomycin for injection.

AUTHORITY: 21 U.S.C. 357.

Subpart A—Bulk Drugs

§ 450.10a Sterile bleomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile bleomycin sulfate is the amorphous sulfate salt of bleomycin. Bleomycin has been separated into several similar glyco-peptide molecules. It is a cream-colored powder that is so purified and dried that:

(i) Its potency is not less than 1.5 units and not more than 2.0 units of bleomycin per milligram. If it is packaged for dispensing, the content of the ampoule or vial is not less than 90 percent and not more than 120 percent of the number of units of bleomycin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 6.0 percent.

(vii) Its pH in an aqueous solution containing 10 units per milliliter is not less than 4.5 and not more than 6.0.

(viii) Its copper content is not greater than 0.1 percent.

(ix) Its content of various bleomycins is as follows: Bleomycin A₂ is not less than 55 percent and not more than 70 percent; bleomycin B₂ is not less than 25 percent and not more than 32 percent; bleomycin B₄ is not more than 1 percent. Bleomycins A₂ and B₂ should comprise not less than 85 percent of the total bleomycins.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, copper, content of various bleomycins, and identity.

(ii) Samples required:

(a) For all tests except sterility: A minimum of 20 immediate containers.

(b) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1M potassium phosphate buffer, pH 7.0 (solution 16), to provide a stock solution of convenient concentration; if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents. Dilute the sample thus obtained with solution 16 to provide a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 16 to the reference concentration of 0.04 unit of activity per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 0.5 unit of bleomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, using the total contents of 2 or 3 vials.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 units per milliliter.

(8) *Copper content*—(i) *Reagents.* Dissolve 10 milligrams of zinc dibenzylthiocarbamate in 100 milliliters of carbon tetrachloride.

(ii) *Preparation of standard copper solution.* Accurately weigh 1.965 grams of cupric sulfate pentahydrate and transfer to a 1-liter volumetric flask. Dissolve the material in 0.1N hydrochloric acid, dilute to volume with 0.1N hydrochloric acid and mix well. Transfer 3 milliliters of this stock solution to a 1-liter volumetric flask, dilute to volume with 0.1N hydrochloric acid, and mix well. This standard copper solution contains 0.0015 milligram of copper per

milliliter. Transfer 10 milliliters of the standard copper solution to a 60-milliliter separatory funnel.

(iii) *Preparation of the sample.* Accurately weigh approximately 15 milligrams of sample into a 60-milliliter separatory funnel. Dissolve the sample in 10 milliliters of 0.1N hydrochloric acid.

(iv) *Procedure.* To the separatory funnels containing the sample solution and standard copper solution, add 10 milliliters of the zinc

dibenzylthiocarbamate solution and shake the funnels vigorously for 1 minute. Allow the phases to separate. Filter the carbon tetrachloride phase (lower phase) through 1 gram of anhydrous sodium sulfate to remove excess water. Using a suitable spectrophotometer equipped with 1-centimeter cells, and carbon tetrachloride as a blank, measure the absorbance of the standard copper solution and the sample solution at 435 nanometers. Calculate the percent copper as follows:

$$\text{Percent copper} = \frac{\text{Absorbance of sample solution} \times 1.5}{\text{Absorbance of standard copper solution} \times \text{Sample weight in milligrams}}$$

(9) *Content of various bleomycin fractions.* Proceed as directed in §436.339 of this chapter.

(10) *Identity test.* Proceed as directed in §436.211 of this chapter, using the method described in paragraph (b)(1) of that section, using a 1 percent mixture.

[40 FR 52005, Nov. 7, 1975; 40 FR 53998, Nov. 20, 1975, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51913, Nov. 15, 1983; 50 FR 19920, May 13, 1985]

§ 450.20 Dactinomycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Dactinomycin is a bright-red compound that is so purified and dried that:

(i) Its dactinomycin content is not less than 900 micrograms of dactinomycin per milligram, calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 15 percent.

(iii) Its absorptivity at 445 nanometers is not less than 0.95 and not more than 1.03 times that of the dactinomycin working standard at the same wavelength. Its absorbance at 240 nanometers is not less than 1.3 and not more than 1.5 times its absorbance at 445 nanometers.

(iv) It is crystalline.

(v) It passes the identity test for dactinomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of

§432.5(b) of this chapter, and in addition each package shall bear on its label the statement "Protect from light and excessive heat."

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(ii) Samples required: 16 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content.* Proceed as directed in §436.331 of this chapter, preparing the sample and calculating the dactinomycin content as follows:

(i) *Preparation of sample solution.* Accurately weigh a sufficient amount of the sample to obtain a solution containing approximately 0.25 milligram per milliliter of dactinomycin in mobile phase.

(ii) *Calculations.* Calculate the micrograms of dactinomycin per milligram of sample as follows:

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

where:

A_u = Area of the dactinomycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the dactinomycin peak in the chromatogram of the dactinomycin working standard;

P_s = Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Absorptivity*—(i) *Procedure.* Accurately weigh approximately 15 milli-

grams of the sample "as is" and 15 milligrams of the working standard dried as directed in § 436.200(a) of this chapter. Transfer each weighing to separate 100-milliliter volumetric flasks. Dissolve the material and bring to volume with spectrophotometric-grade methyl alcohol. Mix well. Pipette 5.0 milliliters of each solution into separate 25-milliliter volumetric flasks, dilute to volume with spectrophotometric-grade methyl alcohol. Mix well. Using a suitable spectrophotometer and 1-centimeter absorption cells, determine the absorbance of the sample solution at the 240-nanometer and at the 445-nanometer absorption peaks (the exact position of the peaks should be determined for the particular instrument used). Determine the absorbance of the standard at the 445-nanometer absorption peak.

(ii) *Calculations.* Calculate the relative absorptivity and the ratio for the absorbances of the sample as follows:

$$\text{Relative absorptivity at 445 nanometers} = \frac{A_2 \times \text{milligrams of standard} \times \text{potency of the standard in micrograms per milligram}}{A_3 \times \text{milligrams of sample} \times (100 - M) \times 10}$$

$$\text{Ratio for the absorbances of the sample at 240 and 445 nanometers} = \frac{A_1}{A_2}$$

where:

A_1 =Absorbance at 240 nanometers for the sample;

A_2 =Absorbance at 445 nanometers for the sample;

A_3 =Absorbance at 445 nanometers for the standard;

M =Percent moisture in the sample.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the dactinomycin working standard.

[49 FR 6092, Feb. 17, 1984, as amended at 49 FR 24018, June 11, 1984; 50 FR 19675, May 10, 1985]

§ 450.22 Daunorubicin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride is the monohydrochloride salt of (1*s*,3*s*)-3-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthaceny-3-amino-2,3,6-trideoxy- α -L-*lyxo*-hexopyranoside. It is a red-orange, hygroscopic powder. It is so purified and dried that:

(i) Its potency is not less than 842 micrograms and not more than 1,030 micrograms of daunorubicin per milligram.

(ii) Its moisture content is not more than 3.0 percent.

(iii) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It is crystalline.

(v) It passes the identity test for daunorubicin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples required: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Daunorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood. Wear rubber gloves, protective gowns, head coverings, and protective eye goggles when handling dry powders. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, promptly wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite solution.

(1) *Potency.* Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography shall be conclusive.

(i) *High-pressure liquid chromatography.* Proceed as directed in § 436.322 of this chapter, except in lieu of the mobile phase and pH described in paragraph (b)(2) of that section, use a mixture of water: acetonitrile (62:38) adjusted to pH 2.2±0.2 with phosphoric acid. Prepare the sample and standard solutions and calculate the daunorubicin content as follows:

(a) *Preparation of sample and working standard solutions.* Accurately weigh approximately 25 milligrams of the sample and of the daunorubicin working standard and dissolve each in 25 milliliters of the internal standard solution prepared as directed in § 436.322(b)(3) of this chapter.

(b) *Calculations.* Calculate the daunorubicin content as follows:

$$\text{Micrograms of daunorubicin per milligram} = \frac{R_u \times W_s \times P \times 100}{R_s \times W_u \times (100 - M)}$$

where:

R_u =Area of the daunorubicin sample peak/
Area of the internal standard peak;

R_s =Area of the daunorubicin standard peak/
Area of the internal standard peak;

W_s =Weight of the daunorubicin working standard in milligrams;

W_u =Weight of the sample in milligrams;

M =Moisture content of the sample in percent;

P =Potency of the daunorubicin working standard in micrograms per milligram.

(ii) *Microbiological turbidimetric assay for daunorubicin—(a) Preparation of working standard stock solutions and standard response line concentrations.*

Dissolve an accurately weighed portion of the working standard with sufficient 0.054M sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter. The working standard stock solution may be stored under refrigeration for 1 week. Further dilute an aliquot of the stock solution with solution 18 to obtain standard response line concentrations of 4, 8, and 16 micrograms of daunorubicin activity per milliliter. The 8-micrograms-per-milliliter concentration is the reference concentration of the assay.

(b) *Preparation of sample solution.* Dissolve an accurately weighed portion of the sample with sufficient 0.054M sodium phosphate buffer, pH 6.9 (solution 18), as described in § 436.101(a)(18) of this chapter, to obtain a stock solution containing 1 milligram of daunorubicin activity per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 18 to the reference concentration of 8 micrograms of daunorubicin activity per milliliter (estimated).

(c) *Procedure for assay.* Place 1.0 milliliter of each concentration of the standard response line and of the sample solution in each set of replicate tubes (as described in § 436.100(b)(1) of this chapter). Eighteen tubes are used for the three-point standard response line and six for each sample. To each tube, add 9 milliliters of medium 3 (as listed in § 436.102(b)(3) of this chapter), inoculated with 2 milliliters of a suspension of test organism I per liter of medium 3. The suspension of test organism I is prepared as described in § 436.103 of this chapter, except incubate the slants and Roux bottle for 16 to 18 hours at 37° C. Place the inoculated tubes immediately in a water bath at 37° C for approximately 3 hours.

The absorbance value for the growth control should be approximately 0.70-0.75 and the absorbance values for the 16 and 4 micrograms per milliliter standard doses should be approximately 0.25-0.35 and 0.55-0.65, respectively. An adjustment of the inoculum may be necessary in order to obtain absorbance values to these approximate levels in a 3-hour time period. Remove the tubes from the water bath and add 0.5 milliliter of a 12-percent formaldehyde solution to each tube. Determine the absorbance value of each tube in a suitable spectrophotometer, at a wavelength of 530 nanometers. Set the instrument at zero absorbance with an uninoculated blank composed of the same amounts of medium 3, solution 18, and formaldehyde used in the assay.

(d) *Estimation of potency.* Estimate the potency of the sample as follows: Using the three x values and the three corresponding y values, calculate Σx , Σx^2 , $(\Sigma x)^2$, Σy and Σxy . Calculate b, the slope (regression coefficient), and a, the Y-intercept of the standard response line by the following equations:

$$b = \frac{n \sum xy - (\sum x)(\sum y)}{n \sum x^2 - (\sum x)^2}$$

$$a = \frac{\sum y - b \sum x}{n}$$

Standard doses (micrograms per milliliter)	16.0	8.0	4.0	n = 3
Log doses (x)	1.20412	0.90309	0.60206	$\Sigma x = 2.70927$
x^2	1.4499	0.81557	0.36248	$(\Sigma x)^2 = 7.34014$
Absorbance readings	0.247	0.483	0.583	$\Sigma x^2 = 2.62795$
	0.236	0.414	0.584	
	0.241	0.446	0.574	
	0.236	0.423	0.555	
	0.233	0.416	0.578	
	0.243	0.413	0.559	
Mean responses (y)	0.239	0.433	0.572	$\Sigma y = 1.244$
xy	0.28778	0.39104	0.34438	$\Sigma xy = 1.0232$

where:

n=Number of standard doses;
 x=Logarithm of the concentration in micrograms per milliliter of each dose of the standard curve;

y=Mean response of the six absorbance values for each dose of the standard.

Calculate the concentration of the sample solution X corresponding to the observed mean response of the sample solution Y by the following equation:

$$x = \text{antilog} \frac{Y - a}{b}$$

where:

X=The concentration of the sample solution in micrograms per milliliter;

Y=The mean response of the six absorbance values for reference concentration sample solutions.

Calculate the potency of the daunorubicin sample as follows:

$$\text{Potency of daunorubicin sample} = \frac{X \times F}{W}$$

in micrograms per milligram

where:

F=125, the appropriate dilution factor of the daunorubicin sample;

W=Weight of sample in milligrams.

The following example illustrates the mathematical calculations of the potency of a sample solution:

Mean response, Y, of sample solution=0.405.

$$b = \frac{3(1.0232) - (2.70927)(1.244)}{3(2.62795) - (2.70927)^2} = -0.553$$

$$a = \frac{1.244 - (-0.553)(2.70927)}{3} = 0.914$$

$$\text{Calculated concentration, X, sample solution} = \text{antilog} \frac{0.405 - 0.914}{-0.553} = 8.32 \text{ micrograms per milliliter}$$

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1)(i) of this section compares qualitatively to that of the daunorubicin working standard.

[45 FR 75195, Nov. 14, 1980]

§ 450.24 Doxorubicin hydrochloride.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Doxorubicin hydrochloride is the monohydrochloride salt of (8S, 10S)-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione. It is a red-orange, almost completely odorless, hygroscopic powder. It is so purified and dried that:

(i) Its doxorubicin hydrochloride content is not less than 970 micrograms and not more than 1,020 micrograms of doxorubicin hydrochloride per milligram on the anhydrous and solvent free basis.

(ii) Its total solvent residue (as acetone and alcohol) is not more than 2.5 percent.

(iii) It contains no depressor substances.

(iv) Its moisture content is not more than 4.0 percent.

(v) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 4.0 and not more than 5.5.

(vi) It is crystalline.

(vii) It passes the identity test for doxorubicin.

(viii) The total of any impurities detected by high-pressure liquid chromatography assay is not more than 3.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each request shall contain:

(i) Results of tests and assays on the batch for doxorubicin hydrochloride content, solvent residue, depressor substances, moisture, pH, crystallinity, identity, and total impurities.

(ii) Samples required: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography).* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4.6-millimeter X 25-centimeter column packed with micro-particulate (5 to 10 micrometers in diameter) packing material, such as trimethylsilane chemically bonded to porous silica, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Prepare a suitable mixture of water, acetonitrile, methanol, and phosphoric acid (540:290:170:2). Dissolve 1 gram of sodium lauryl sulfate in 1,000 milliliters of this solution, adjust with 2*N* sodium hydroxide to a pH of 3.6±0.1. Filter through a suitable

filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighed quantity of doxorubicin hydrochloride working standard in mobile phase to obtain a solution having a known concentration of 0.1 milligram of doxorubicin hydrochloride per milliliter.

(B) *Sample solution.* Transfer approximately 20 milligrams of sample, accurately weighed, to a 200-milliliter volumetric flask, add mobile phase to volume, and mix. This yields a solution containing 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(C) *Resolution test solution.* Use either of the following preparation methods:

(1) To 2 milliliters of a 1.0 milligram per milliliter solution of doxorubicin hydrochloride, add 20 microliters of 1*N* hydrochloric acid. Hold for 30 minutes at 95 °C in an oil bath.

(2) Dissolve about 10 milligrams of doxorubicin hydrochloride in 5 milliliters of water, add 5 milliliters of phosphoric acid, and allow to stand for about 30 minutes. Adjust with 2*N* sodium hydroxide (about 37 milliliters) to a pH of 2.6±0.1, add 15 milliliters of acetonitrile and 10 milliliters of methanol, mix, and filter. (Note: Portions of this solution may be frozen until needed, then thawed and mixed before use.)

(3) The procedures in paragraphs (b)(1)(ii)(C)(1) and (b)(1)(ii)(C)(2) of this section generate doxorubicinone, the aglycone of doxorubicin. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* The asymmetry factor (A_s) for the doxorubicin peak measured at a point 5 percent of the peak height is not less than 0.7 and not more than 1.2.

(B) *Efficiency of the column.* The absolute column efficiency (h_r) is satisfactory if it is not greater than 10.0, equivalent to 2,500 theoretical plates for a 25-centimeter column of 10-micrometer particles.

(C) *Resolution.* The resolution (R) between the peaks of doxorubicin and doxorubicinone (generated in situ) is satisfactory if it is not less than 5.5.

(D) *Capacity factor.* The capacity factor (k) for doxorubicin is satisfactory if it is in the range between 1.0 and 5.0.

(E) *Coefficient of variation.* The coefficient of variation (*relative standard of deviation* in percent) of 5 replicate injections is satisfactory if it is not more than 1.0 percent. If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of doxorubicin hydrochloride per milligram of sample as follows:

$$\begin{array}{l} \text{Micrograms of} \\ \text{doxorubicin} \\ \text{hydrochloride} \\ \text{per milligram} \end{array} = \frac{A_U \times P_S \times 100}{A_S \times C_U \times (100 - m - X)}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter;

C_U = Milligrams of the sample per milliliter of sample solution;

m = Percent moisture content of the sample; and

X = Percent solvent residue determined as directed in paragraph (b)(2) of this section.

(2) *Residue solvent (as acetone and alcohol)*—(i) *Standard preparation.* Transfer to a 100-milliliter volumetric flask about 200 milligrams of acetone, 300 milligrams of dehydrated alcohol, and 1,000 milligrams of dioxane, each accurately weighed, and mix. Dilute with water to volume, and mix. Transfer 5.0 milliliters of the resulting solution to a 50-milliliter volumetric flask, dilute with water to volume, and mix. This solution contains about 0.2 milligram of acetone, 0.3 milligram of alcohol, and 1 milligram of dioxane per milliliter.

(ii) *Solvent.* Transfer about 100 milligrams of dioxane, accurately weighed to a 100-milliliter volumetric flask, dilute with water to volume, and mix.

(iii) *Test preparation.* Dissolve about 200 milligrams of doxorubicin hydrochloride sample in 3.0 milliliters of solvent.

(iv) *Chromatographic system* (see *United States Pharmacopeia (U.S.P.) Chromatography (621)*). The gas chromatograph is equipped with a flame-ionization detector and a 4-millimeter X 2-meter column packed with 8-percent liquid phase G16 (see U.S.P. Chromatographic Reagents—Phases) on 100- to 120-mesh support S1AB (potassium hydroxide-washed) (see U.S.P. Chromatographic Reagents—Supports). The column is maintained at about 60 °C, and helium is used as the carrier gas. Adjust the column temperature and carrier gas flow rate so that dioxane elutes in about 6 minutes. Chromatograph the standard preparation, and record the peak responses as directed under procedure; the resolution (*R*) between adjacent peaks is not less than 2.0; the relative standard deviations of the ratios of the peak responses of the acetone and dioxane peaks and of the alcohol and dioxane peaks for replicate injections is not more than 4.0 percent; and the tailing factor for the alcohol peak is not more than 1.5.

(v) *Procedure.* (Note: Use peak areas where peak responses are indicated.) Separately inject equal volumes (about 1 microliter) of the standard preparation and the test preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.2 for acetone, 0.5 for alcohol, and 1.0 for dioxane. Calculate the percentage, by weight, of acetone and alcohol, respectively, in the sample as follows:

$$X = \text{Percent acetone or alcohol} = \frac{100(C_A C_D)(D_U W_U)(R_U/R_S)}{100(C_A C_D)(D_U W_U)(R_U/R_S)}$$

where:

C_A = Concentration of acetone or alcohol in the standard preparation in milligrams per milliliter;

C_D = Concentration of dioxane in the standard preparation in milligrams per milliliter;

D_U = Total quantity of dioxane in the test preparation, in milligrams;

W_U = Quantity of doxorubicin hydrochloride taken to prepare the test preparation, in milligrams;

R_U = Response ratio of the analyte peak (acetone or alcohol) to the dioxane peak obtained from the test preparation; and

R_S = Response ratio of the analyte peak (acetone/alcohol) to the dioxane peak obtained from the standard preparation.

The total of acetone and alcohol is not greater than 2.5 percent. Use the result obtained to calculate the doxorubicin hydrochloride content of the sample on the solvent-free basis.

(3) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the doxorubicin hydrochloride working standard.

(8) *Chromatographic purity.* Proceed as directed in paragraph (b)(1) of this section, except prepare the sample solution by dissolving the sample to be tested in mobile phase to obtain a solution containing approximately 0.5 milligram of doxorubicin hydrochloride per milliliter. Calculate the percentage of impurities as follows:

$$\text{Percent total impurities} = \frac{(100 S)}{(S + r)}$$

where:

S = The sum of the responses of the minor component peaks; and

r = The response of the major doxorubicin hydrochloride peak.

The total related impurities detected is not more than 2.0 percent.

[41 FR 14184, Apr. 2, 1976; 41 FR 15844, Apr. 15, 1976, as amended at 42 FR 43063, Aug. 26, 1977; 43 FR 44836, Sept. 29, 1978; 47 FR 9396, Mar. 5, 1982; 47 FR 23710, June 1, 1982; 50 FR 19676, May 10, 1985; 53 FR 37292, Sept. 26, 1988; 59 FR 9639, Mar. 1, 1994]

§ 450.30 Idarubicin hydrochloride.

(a) *Requirements for certification—(1) Standards of identity, strength, quality,*

and purity. Idarubicin hydrochloride is the monohydrochloride salt of 5,12-Naphthacenedione,9-acetyl-7-[(3-amino-2,3,6-trideoxy- α -*L*-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11-trihydroxy-(7*S*-*cis*). It is an orange-red powder. It is so purified and dried that:

(i) Its idarubicin hydrochloride content is not less than 960 micrograms and not more than 1,030 micrograms of idarubicin hydrochloride per milligram on the anhydrous basis.

(ii) Its moisture content is not more than 5.0 percent.

(iii) The pH of an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 6.5.

(iv) It is crystalline.

(v) The level of any individual impurity detected by high-performance liquid chromatography (HPLC) assay is not more than 1.0 percent.

(vi) The total of all detected impurities is not more than 3.0 percent.

(vii) It passes the identity test for idarubicin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for idarubicin hydrochloride content, solvent residues, moisture, pH, crystallinity, related individual thin-layer chromatography and HPLC impurities, total impurities, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 14 packages, each containing approximately 40 milligrams.

(b) *Tests and methods of assay.* Idarubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders into a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Potency (HPLC).* Proceed as directed in § 436.216 of this chapter, using

ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a 4.6-millimeter by 25-centimeter column packed with microparticulate (5 to 10 micrometers in diameter) packing material such as trimethylsilane chemically bonded to porous silica, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. The retention time for idarubicin hydrochloride is between 14 and 16 minutes. The retention time for the resolution compound

4-demethoxydaunorubicinone (generated in situ) is between 6 and 9 minutes. Mobile phase, diluent, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Prepare a suitably sized quantity of a mixture of water, acetonitrile, and methanol (540:290:170). Dissolve 1 gram of sodium lauryl sulfate and 2 milliliters of 85 percent phosphoric acid per liter of this solution. Adjust with 2 *N* sodium hydroxide to a pH of 3.6±0.1. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Diluent.* Prepare as mobile phase, excluding the sodium lauryl sulfate.

(iii) *Preparation of working standard solution.* Dissolve an accurately weighed quantity of idarubicin hydrochloride working standard in diluent to obtain a solution having a known concentration of 0.5 milligram of idarubicin hydrochloride per milliliter.

(iv) *Sample solution.* Transfer approximately 50 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask, add diluent to volume, and mix. This yields a solution containing 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(v) *Resolution test solution.* To 2 milliliters of a 1.0 milligram per milliliter aqueous solution of idarubicin hydrochloride, add 20 microliters of 1 *N* hydrochloric acid. Hold for 30 minutes at 95 C in an oil bath. This procedure generates the aglycone of idarubicin, 4-demethoxydaunorubicinone. Transfer 1.0 milliliter of this solution to a 10-milliliter volumetric flask, add diluent

to volume, and mix. Use this solution to determine the resolution requirement for the chromatographic system.

(vi) *System suitability requirements*—
(A) *Asymmetry factor*. The asymmetry factor (A_s), measure data point 5 percent of the peak height from the baseline, is satisfactory if it is not less than 0.85 and not more than 1.1.

(B) *Efficiency of the column*. The absolute efficiency (h_r) is satisfactory if it is not more than 10.0 for the idarubicin hydrochloride peak, equivalent to 4,500 theoretical plates for a 25-centimeter column of 6-micrometer particles.

(C) *Resolution factor*. The resolution factor (R_s) between the peak for idarubicin and 4-demethoxydaunorubicinone (generated *in situ*) is satisfactory if it is not less than 9.5.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation (S_R in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor*. The capacity factor (k') for idarubicin hydrochloride is satisfactory if it is not less than 5 and not more than 15. If the system suitability parameters have been met, proceed as described in § 436.216(b) of this chapter.

(vii) *Calculations*. Calculate the micrograms of idarubicin hydrochloride per milligram of sample as follows:

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

where:

A_u =Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_s =Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter;

C_u =Milligrams of idarubicin hydrochloride sample per milliliter of sample solution;

m =Percent moisture content of the sample.

(2) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solu-

tion containing 5 milligrams per milliliter.

(4) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

(5) *HPLC impurities*. Proceed as directed in paragraph (b)(1) of this section. Calculate the percentage of impurities as follows:

$$\text{Percent individual impurity} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC impurities} = \frac{A \times 100}{A_t}$$

where:

A_i =Area of the individual impurity peak;

A =The sum of areas of all peaks minus the area due to the idarubicin hydrochloride peak and solvent peak; and

A_t =The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 1.0 percent potassium bromide disc prepared as directed in § 436.211(b)(1).

[58 FR 26664, May 4, 1993]

§ 450.40 Plicamycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Plicamycin is a yellow compound and is so purified and dried that:

(i) Its plicamycin content is not less than 900 micrograms of plicamycin per milligram calculated on an anhydrous basis.

(ii) Its loss on drying is not more than 8 percent.

(iii) Its pH in an aqueous solution containing 0.5 milligram per milliliter is not less than 4.5 nor more than 5.5.

(iv) Its absorptivity on the anhydrous basis at the absorption maximum of 278 millimicrons is 100±5 percent of that of the plicamycin standard similarly treated.

(v) It gives a positive result to the identity tests for plicamycin.

(vi) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label the statement “Store below 10° C. (50° F.)”.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for plicamycin content, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required on the batch: 2 packages, each containing not less than 100 milligrams; and 3 packages, each containing not less than 50 milligrams.

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Solutions should not be pipetted by mouth. Plicamycin is hygroscopic and care should be exercised during storage and weighing samples. Samples should be stored at 10° C. or less in a sealed, light-resistant container with a desiccant. Dispose of all waste material by dilution with larger volumes of trisodium phosphate solution.

(1) *Plicamycin content.* Proceed as directed in §436.341 of this chapter, preparing the sample and calculating the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flask and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the micrograms of plicamycin per milligram of sample as follows:

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

where:

A_u = Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s = Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter;

C_u = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Loss on drying.* Proceed as directed in §436.200(g) of this chapter.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 0.5 milligram of plicamycin per milliliter. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(4) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 10 milligrams each of the sample and standard (dried as described in §436.200(g) of this chapter), accurately weighed, in 50 milliliters of absolute methanol. Transfer 5-milliliter portions into 100-milliliter volumetric flasks and dilute to volume with 0.01N hydrochloric acid in methanol prepared by diluting 20 milliliters of 0.5N aqueous hydrochloric acid to 1 liter with absolute methanol. Using a suitable spectrophotometer and 0.01N hydrochloric acid in methanol as the blank, scan the absorption spectrum between the wavelengths of 220 millimicrons and 400 millimicrons. Determine the absorbance of each solution at the absorption maximum near 278 millimicrons. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample solution} \times \text{milligrams of standard} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard solution} \times \text{milligrams of sample} \times 10}$$

(5) *Identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the plicamycin working standard.

(6) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19145, May 30, 1974, as amended at 49 FR 5097, Feb. 10, 1984; 49 FR 24018, June 11, 1984]

§ 450.45 Mitomycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Mitomycin is 7-amino-9a-methoxymitosane. It is a blue-violet compound that is soluble in water, methanol, acetone, butyl acetate, and cyclohexanone. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in a solution containing 5 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) When calculated on the anhydrous basis, its absorptivity at 357 nanometers is not less than 95 percent and not more than 105 percent of that of the mitomycin working standard similarly treated.

(vi) It gives a positive identity test for mitomycin.

(vii) It is crystalline.

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the 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, absorptivity, identity, and crystallinity.

(ii) Samples required: Five packages, each containing approximately 100 milligrams.

(b) *Tests and methods of assay—(1) Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 1.0 microgram of mitomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Absorptivity*. Determine the absorbance of the sample and standard solution in the following manner: Place an accurately weighed portion of approximately 25 milligrams of mitomycin into a 50-milliliter volumetric flask. Dissolve and dilute to volume with absolute methanol. Further dilute an aliquot with absolute methanol to 0.005 milligram of mitomycin per milliliter. Using a suitable spectrophotometer equipped with a 1-centimeter quartz cell and absolute methanol as the blank, determine the absorbance of the sample and standard solutions at 357 nanometers. Calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{percent mitomycin content of standard} \times 100}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where:

m=percent moisture in the sample.

ple preparation method described in paragraph (b)(2) of that section.

(6) *Identity*. Proceed as directed in § 436.211 of this chapter, using the sam-

(7) *Crystallinity*. Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19145, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]

Subpart B [Reserved]

Subpart C—Injectable Dosage Forms

§ 450.210 Sterile bleomycin sulfate.

The requirements for certification and the tests and methods of assay for sterile bleomycin sulfate packaged for dispensing are described in § 450.10a.

[40 FR 52006, Nov. 7, 1975]

§ 450.220 Dactinomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Dactinomycin for injection is a dry mixture of dactinomycin and mannitol. Each container contains 0.5 milligram of dactinomycin. Its dactinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of dactinomycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 4.0 percent. Its pH is not less than 5.5 and not more than 7.5. The dactinomycin used conforms to the standards prescribed by § 450.20(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter, and in addition each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container the statement "Protect from light and excessive heat".

(ii) On the outside wrapper or container and the immediate container the statement "For hospitalized patients only".

(3) *Requests for certification; samples*. In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The dactinomycin used in making the batch for dactinomycin content, loss on drying, absorptivity, crystallinity, and identity.

(b) The batch for dactinomycin content, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(a) The dactinomycin used in making the batch: 10 containers each containing not less than 40 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 20 immediate containers.

(2) For sterility testing: 20 immediate containers.

(b) *Tests and methods of assay*. Dactinomycin is toxic and corrosive. It must be handled with care in the laboratory. Transfer all dry powders in a suitable hood, while wearing rubber gloves. Avoid inhaling fine particles of the powder. Do not pipette by mouth. If any of the substance contacts the skin, wash copiously with soap and water. Dispose of all waste material by dilution with large volumes of trisodium phosphate solution.

(1) *Dactinomycin content*. Proceed as directed in § 436.331 of this chapter, except prepare the sample solution and calculate the dactinomycin content as follows:

(i) *Sample solution*. Reconstitute the vial with 2.0 milliliters of mobile phase. Shake well and filter if necessary.

(ii) *Calculations*. Calculate the dactinomycin content of the vial as follows:

$$\text{Milligrams of dactinomycin per vial} = \frac{A_u \times P_s \times d}{A_s \times 500}$$

where:

A_u =Area of the dactinomycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the dactinomycin peak in the chromatogram of the dactinomycin working standard;

P_s =Dactinomycin activity in the dactinomycin working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, preparing the

sample for test as follows: Use a sufficient number of containers to yield 3 milligrams of dactinomycin. Reconstitute by adding 1.1 milliliters of sterile water for injection to each container. Aseptically pool the resultant solutions from each container. Dilute an accurately measured portion with sufficient diluent 1 to give a concentration of 0.2 milligram of dactinomycin per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

[39 FR 19145, May 30, 1974, as amended at 44 FR 10379, Feb. 20, 1979; 46 FR 16685, Mar. 13, 1981; 46 FR 46313, Sept. 18, 1981; 49 FR 6093, Feb. 17, 1984; 49 FR 15074, Apr. 17, 1984; 49 FR 24018, June 11, 1984; 50 FR 1504, Jan. 11, 1985; 50 FR 19676, May 10, 1985]

§ 450.222 Daunorubicin hydrochloride for injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Daunorubicin hydrochloride for injection is a freeze-dried powder whose components are daunorubicin hydrochloride and mannitol. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of daunorubicin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its moisture content is not more than 3.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The daunorubicin hydrochloride used conforms to the standards prescribed by § 450.22(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The daunorubicin hydrochloride used in making the batch for potency, moisture, pH, crystallinity, and identity.

(b) The batch for potency, sterility, pyrogens, depressor substances, moisture, pH, and identity.

(ii) Samples required:

(a) The daunorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 34 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Daunorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood. Wear rubber gloves, protective gowns, head coverings, and protective eye goggles when handling dry powders. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with larger volumes of sodium hypochlorite solution.

(1) *Daunorubicin content (high-pressure liquid chromatography).* Proceed as directed in § 436.322 of this chapter, preparing the sample and standard solutions and calculating the daunorubicin content as follows:

(i) *Preparation of working standard solution.* Accurately weigh approximately 25 milligrams of the daunorubicin working standard and dissolve in 25 milliliters of the internal standard solution prepared as directed in § 436.322(b)(3) of this chapter.

(ii) *Preparation of sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate-sized volumetric flask with a sufficient amount of internal standard solution prepared as directed in § 436.322(b)(3) of this chapter, to obtain a concentration of 1.0 milligram of daunorubicin per milliliter.

(iii) *Calculations.* Calculate the daunorubicin content as follows:

$$\text{Daunorubicin content per vial} = \frac{R_u \times W_s \times V \times P}{R_s \times 25 \times 1,000}$$

in milligrams

where:

$$R_u = \frac{\text{Area of the daunorubicin sample peak}}{\text{Area of the internal standard peak}}$$

$$R_s = \frac{\text{Area of the daunorubicin standard peak}}{\text{Area of the internal standard peak}}$$

W_s = Weight of the daunorubicin working standard in milligrams;

V = Volume in milliliters of the internal standard solution added to the vials;

P = Potency of the daunorubicin working standard in micrograms per milligram.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in § 436.32(a) of this chapter, using a solution containing 2.25 milligrams of daunorubicin per milliliter.

(4) *Depressor substances*. Proceed as directed in § 436.35 of this chapter.

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in paragraph (d)(4) of that section.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling.

(7) *Identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the daunorubicin working standard.

[45 FR 75198, Nov. 14, 1980, as amended at 50 FR 47214, Nov. 15, 1985]

§ 450.224 Doxorubicin hydrochloride injectable dosage forms.

§ 450.224a Doxorubicin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Doxorubicin hydrochloride for injection is a freeze-dried powder whose components are doxorubicin hydrochloride and lactose. It may also contain methylparaben. Its doxorubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of doxorubicin hydrochloride that it is

represented to contain. It is sterile. It contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 4.5 and not more than 6.5. It passes the identity test. The doxorubicin hydrochloride used conforms to the standards prescribed by § 450.24(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The doxorubicin hydrochloride used in making the batch for doxorubicin hydrochloride content, residue solvents, depressor substances, moisture, pH, crystallinity, identity, and total related impurities.

(b) The batch for doxorubicin hydrochloride content, sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples required:

(a) The doxorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 34 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*. Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood while wearing rubber gloves. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography)*. Proceed as directed in § 450.24(b)(1), preparing the sample solution and calculating the doxorubicin hydrochloride content as follows:

(i) *Sample solution*. Prepare the sample solution by rinsing the contents of the vial into an appropriate sized volumetric flask with sufficient mobile phase to obtain a concentration of 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(ii) *Calculations*. Calculate the doxorubicin hydrochloride content per vial as follows:

$$\text{Milligrams of doxorubicin hydrochloride per vial} = \frac{A_U \times P_S \times d}{A_S \times 1,000}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Bacterial endotoxins*. Proceed as directed in the United States Pharmacopeia (U.S.P.) Bacterial Endotoxin Test, using a solution of doxorubicin hydrochloride for injection containing 1.1 milligrams of doxorubicin hydrochloride per milliliter. The specimen under test contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride.

(4) [Reserved]

(5) *Moisture*. Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in paragraph (d)(4) of that section.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling, except in lieu of saline use distilled water.

(7) *Identity*. The high-pressure liquid chromatogram of the sample deter-

mined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the doxorubicin hydrochloride working standard.

[41 FR 14185, Apr. 2, 1976, as amended at 43 FR 44837, Sept. 29, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19676, May 10, 1985. Redesignated at 53 FR 37292, Sept. 26, 1988; 59 FR 9641, Mar. 1, 1994]

§ 450.224b Doxorubicin hydrochloride injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Doxorubicin hydrochloride injection is an aqueous solution of doxorubicin hydrochloride in an isosmotic diluent. Each milliliter contains doxorubicin hydrochloride equivalent to 2 milligrams of doxorubicin hydrochloride. Its doxorubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams it is represented to contain. It is sterile. It contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride. Its pH is not less than 2.5 and not more than 3.5. It passes the identity test. The doxorubicin hydrochloride used conforms to the standards prescribed by § 450.24(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The doxorubicin hydrochloride used in making the batch for doxorubicin hydrochloride content, residue solvents, depressor substances, moisture, pH, crystallinity, identity, and total related impurities.

(B) The batch for doxorubicin hydrochloride content, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples required:

(A) The doxorubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(B) The batch:

(1) For all tests except sterility: A minimum of 34 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Doxorubicin hydrochloride is toxic. It must be handled with care in the laboratory. Solutions should not be pipetted by mouth. Transfer all dry powders in a suitable hood while wearing rubber gloves. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of sodium hypochlorite (bleach) solution.

(1) *Doxorubicin hydrochloride content (high-performance liquid chromatography).* Proceed as directed in § 450.24(b)(1), preparing the sample solution and calculating the doxorubicin hydrochloride content as follows:

(i) *Sample solution.* Dilute an accurately measured volume of sample equivalent to not less than 2 milligrams of doxorubicin hydrochloride, quantitatively with mobile phase to obtain a solution containing 0.1 milligram of doxorubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the milligrams of doxorubicin hydrochloride per milliliter of sample as follows:

$$\text{Milligrams of doxorubicin hydrochloride per milliliter} = \frac{A_U \times P_S \times d}{A_S \times 1,000}$$

where:

A_U = Area of the doxorubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_S = Area of the doxorubicin hydrochloride peak in the chromatogram of the doxorubicin hydrochloride working standard;

P_S = Doxorubicin hydrochloride activity in the doxorubicin hydrochloride working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) [Reserved]

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia (U.S.P.) Bacterial Endotoxin Test, using a test solution prepared by diluting doxorubicin hydrochloride injection with sterile water for injection

to obtain a concentration of 1.1 milligrams of doxorubicin hydrochloride per milliliter. The specimen under test contains not more than 2.2 U.S.P. endotoxin units per milligram of doxorubicin hydrochloride.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(6) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section, compares qualitatively to that of the doxorubicin hydrochloride working standard.

[53 FR 37292, Sept. 26, 1988, as amended at 59 FR 9641, Mar. 1, 1994]

§ 450.230 Idarubicin hydrochloride for injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Idarubicin hydrochloride for injection is a lyophilized mixture of idarubicin hydrochloride and lactose. Its idarubicin hydrochloride content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of idarubicin hydrochloride that it is represented to contain. It is sterile. It contains not more than 8.93 U.S.P. endotoxin units per milligram of idarubicin hydrochloride. Its moisture content is not more than 4.0 percent. When reconstituted as directed in the labeling, its pH is not less than 5.0 and not more than 7.0. It passes the identity test. The idarubicin hydrochloride used conforms to the standards prescribed by § 450.30(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The idarubicin hydrochloride used in making the batch for idarubicin hydrochloride content, solvent residues, moisture, pH, crystallinity, related individual thin layer chromatography and high-performance liquid chromatography (HPLC) impurities, total impurities, and identity.

(B) The batch for idarubicin hydrochloride content, sterility, bacterial endotoxins, moisture, pH, and identity.

(ii) Samples required if requested by the Director, Center for Drug Evaluation and Research:

(A) The idarubicin hydrochloride used in making the batch: 14 packages, each containing approximately 40 milligrams.

(B) The batch:

(1) For all tests except sterility: A minimum of 34 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Idarubicin hydrochloride is toxic. It must be handled with care in the laboratory. Transfer all dry powders into a suitable hood while wearing rubber gloves. Avoid inhaling fine particles of powder. Solutions should not be pipetted by mouth. If the substance contacts the skin, wash with soap and water. Dispose of all waste material by dilution with large volumes of dilute sodium hypochlorite (bleach) solution.

(1) *Idarubicin hydrochloride content (HPLC).* Proceed as directed in § 450.30(b)(1), preparing the sample solution and calculating the idarubicin hydrochloride as follows:

(i) *Sample solution.* Prepare the sample solution by rinsing the contents of the vial into an appropriate-sized volumetric flask with sufficient diluent to obtain a concentration of 0.5 milligram of idarubicin hydrochloride per milliliter (estimated).

(ii) *Calculations.* Calculate the idarubicin hydrochloride content per vial as follows:

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

where:

A_u =Area of the idarubicin hydrochloride peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the idarubicin hydrochloride peak in the chromatogram of the idarubicin hydrochloride working standard;

P_s =Idarubicin hydrochloride activity in the idarubicin hydrochloride working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(3) *Bacterial endotoxins.* Proceed as directed in the U.S.P. Bacteria endotoxin test. The specimen under test contains not more than 8.93 U.S.P. endotoxin units per milligram of idarubicin hydrochloride.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation method described in § 436.201(d)(4).

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample obtained after reconstituting the drug as directed in the labeling, except use distilled water instead of saline.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the idarubicin hydrochloride working standard.

[58 FR 26665, May 4, 1993]

§ 450.240 Plicamycin for injection.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Plicamycin for injection is a dry mixture of plicamycin and mannitol with or without a suitable buffer substance. Each immediate container contains 2.5 milligrams of plicamycin. Its plicamycin content is satisfactory if it contains not less than 90 percent and not more than 110 percent of the number of milligrams of plicamycin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. It contains no depressor substances. Its pH when reconstituted as directed in the labeling is not less than 5.0 and not more than 7.5. It passes the identity test for plicamycin. The plicamycin used conforms to the standards prescribed by § 450.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, each package shall bear on its label or labeling the following as indicated:

(i) On the outside wrapper or container the statement "Store below 10° C. (50° F.)".

(ii) On the outside wrapper or container and on the immediate container the statement "Mandatory: Before using read enclosed professional information carefully for dosage instructions and warnings".

(iii) On the outside wrapper or container the statement "Warning: For hospital use only. To be used under direct supervision of a physician".

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The plicamycin used in making the batch for plicamycin content, loss on drying, absorptivity, pH, identity, and crystallinity.

(b) The batch for plicamycin content, sterility, pyrogens, moisture, Ph, depressor substances, and identity.

(ii) Samples required:

(a) The plicamycin used in making the batch: 3 packages, each containing not less than 50 milligrams; and 2 packages, each containing not less than 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 21 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Plicamycin is more toxic than the average drug and must be handled with care in the laboratory. Avoid inhaling fine particles of powder. If the substance contacts the skin, wash with soap and water. Plicamycin is hygroscopic and care should be exercised during storage and weighing of samples. Dispose of all waste materials by dilution with larger volumes of trisodium phosphate solution. The samples should be stored at 10° C. or less in a sealed light-resistant container with a desiccant. Solutions should not be pipetted by mouth.

(1) *Plicamycin content.* Proceed as directed in §436.341 of this chapter, except prepare the sample solution and calculate the plicamycin content as follows:

(i) *Preparation of sample solution.* Place approximately 5 milligrams of the sample, accurately weighed, into a 50-milliliter, amber volumetric flash

and dilute to volume with mobile phase and mix.

(ii) *Calculations.* Calculate the plicamycin content of the vial as follows:

$$\text{Milligrams of plicamycin per vial} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

A_u =Area of the plicamycin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the plicamycin peak in the chromatogram of the plicamycin working standard;

P_s =Plicamycin activity in the plicamycin working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use the entire contents of each of the immediate containers tested.

(3) *Pyrogens.* Reconstitute the sample as directed in the labeling and proceed as directed in §436.32(b) of this chapter, using a solution containing 50 micrograms of plicamycin per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter, using the total contents of three to five vials.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using the drug reconstituted as directed in the labeling. Allow the solution to remain in contact with the electrodes until a steady reading is obtained or for 5 minutes.

(6) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(7) *Thin layer chromatography identity test for plicamycin—(i) Equipment—(a) Plates.* Use 20 by 20 centimeter or 15 by 20 centimeter thin layer chromatographic plates coated with Silica Gel Mixture, Chromatographic, U.S.P., to a thickness of 250 microns. Activate the plates by heating at 110° C. for 75 minutes. Place the plates in a desiccator until cooled to room temperature. Plates may be stored in a desiccator for 7 days.

(b) *Chamber (chromatographic).* A suitable chamber, equipped for thin layer chromatography.

(ii) *Preparations of solutions*—(a) *Solvent*. Mix reagent grade chloroform with reagent grade absolute methanol in volumetric proportions of 1:1.

(b) *Spray A*. Mix 50 milliliters of freshly prepared 1.0 percent ferric chloride in water (weight per volume), just before spraying, with 50 milliliters of freshly prepared 1.0 percent potassium ferricyanide in water (weight per volume).

(c) *Spray B*. Dissolve 2.28 grams of periodic acid in 100 milliliters of water. Dilute one volume of this periodic solution with 10 volumes of acetone.

(d) *Spray C*. Dissolve 184 milligrams of benzidine in a solution of 0.6 milliliter of acetic acid, 4.4 milliliters of water, and 95 milliliters of acetone.

(iii) *Preparation of spotting solutions*—(a) *Plicamycin standard solution*. Weigh 5 milligrams of plicamycin working standard and dissolve in 10 milliliters of methanol. Use the solution the same day it is prepared.

(b) *Plicamycin for injection sample solution*. Dilute with methanol to a concentration of 0.5 milligram of plicamycin per milliliter. Centrifuge and use the supernatant for spotting.

(c) *Mannitol reference solution*. Suspend 100 milligrams of mannitol in 5 milliliters of methanol. Centrifuge and use the supernatant for spotting.

(iv) *Procedure*. Fill the chamber to a depth of 0.6 centimeter with freshly prepared solvent. Spot duplicate plates as follows: On a line 2.5 centimeters from the base of the silica gel plate, and at intervals of 2.0 centimeters, spot 100 microliters (in four 25-microliter aliquots) of the standard solution, the sample solution, and the mannitol reference solution. Allow each aliquot to dry before applying subsequent volumes. After all spots are thoroughly dry, place the silica gel plates in the chromatographic chamber and develop by the ascending technique for approximately 60 minutes. Allow several minutes for the plates to air dry. On one plate, locate and record the position of fluorescent spots by examining under long wave ultraviolet light. Apply spray A and record the position of blue spots on the yellow-green background. On the other plate, locate the mannitol by first applying spray B, followed by spray C. The spots appearing white are

mannitol. Measure the distance the solvent front traveled from the starting line and the distance the fluorescent spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The plicamycin standard should have an R_f value of 0.7. If the standard has an R_f value greater than 0.8, the mobility of the standard may be decreased by increasing the ratio of the chloroform to methanol in the solvent to 3:2 or 3:1. Plicamycin appears as a single major component with the same R_f value as the plicamycin standard. It may show trace components at R_f values of about 0.5 and 0.4, and at the origin, which shall not be more intense than those shown by the plicamycin standard.

[39 FR 19145, May 30, 1974, as amended at 40 FR 1512, Jan. 8, 1975; 46 FR 60568, Dec. 11, 1981; 47 FR 9396, Mar. 5, 1982; 48 FR 11427, Mar. 18, 1983; 49 FR 5097, Feb. 10, 1984; 49 FR 24019, June 11, 1984; 50 FR 19676, May 10, 1985]

§ 450.245 Mitomycin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Mitomycin for injection is a dry mixture of mitomycin and mannitol. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of mitomycin that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its moisture content is not more than 5 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.0 and not more than 8.0. It passes the identity test for mitomycin. The mitomycin used conforms to the standards prescribed by § 450.45(a)(1).

(2) *Labeling*. It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples*. In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The mitomycin used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(b) The batch for potency, sterility, pyrogens, depressor substances, moisture, pH, and identity.

(ii) Samples required:

(a) The mitomycin used in making the batch: Five packages, each containing approximately 100 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 25 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 1 to the reference concentration of 1 microgram of mitomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens*. Proceed as directed in §436.32(a) of this chapter, using a solution containing 0.5 milligram of mitomycin per milliliter.

(4) [Reserved]

(5) *Depressor substances*. Proceed as directed in §436.35 of this chapter.

(6) *Moisture*. Proceed as directed in §436.201 of this chapter.

(7) *pH*. Proceed as directed in §436.202 of this chapter using the drug reconstituted as directed in the labeling.

(8) *Identity*. Proceed as directed in §436.310 of this chapter.

[39 FR 19145, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

PART 452—MACROLIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.	
452.10	Erythromycin.
452.15	Erythromycin estolate.
452.25	Erythromycin ethylsuccinate.
452.25a	Sterile erythromycin ethylsuccinate.
452.30a	Sterile erythromycin gluceptate.
452.32a	Sterile erythromycin lactobionate.
452.35	Erythromycin stearate.
452.50	Clarithromycin.
452.60	Azithromycin.
452.75	Troleandomycin.

Subpart B—Oral Dosage Forms

452.110	Erythromycin oral dosage forms.
452.110a	Erythromycin tablets.
452.110b	Erythromycin enteric-coated tablets.
452.110c	Erythromycin capsules.
452.110d	Erythromycin particles in tablets.
452.115	Erythromycin estolate oral dosage forms.
452.115a	Erythromycin estolate tablets.
452.115b	Erythromycin estolate capsules.
452.115c	Erythromycin estolate oral suspension.
452.115d	Erythromycin estolate for oral suspension.
452.115e	Erythromycin estolate for pediatric drops.
452.115f	Erythromycin estolate chewable tablets.
452.115g	Erythromycin estolate and sulfisoxazole acetyl oral suspension.
452.125	Erythromycin ethylsuccinate oral dosage forms.
452.125a	Erythromycin ethylsuccinate chewable tablets.
452.125b	Erythromycin ethylsuccinate oral suspension.
452.125c	Erythromycin ethylsuccinate for oral suspension.
452.125d	Erythromycin ethylsuccinate tablets.
452.125e	Erythromycin ethylsuccinate-sulfisoxazole acetyl for oral suspension.
452.135	Erythromycin stearate oral dosage forms.
452.135a	Erythromycin stearate tablets.
452.135b	Erythromycin stearate oral suspension.
452.135c	Erythromycin stearate for oral suspension.
452.150	Clarithromycin oral dosage forms.
452.150a	Clarithromycin tablets.
452.150b	Clarithromycin granules for oral suspension.