

(D) *Coefficient of variation* (relative standard deviation). The coefficient of variation (S_v in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

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

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

where:

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

A_s =Area of the 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 hydro-

genated 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 1*N* NaOH and then heat the flasks in a boiling water bath for 10 minutes. Cool the flasks in ice water for 3 minutes and acidify the solutions with 2.0 milliliters of 1.2*N* HCl. To each flask add 5.0 milliliters of 0.25 percent ferric chloride reagent, make to volume with distilled water, and mix thoroughly. Transfer the colored solutions to 2.0-centimeter absorption cells and measure the percent light transmission at 530 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:

$$\text{Percent streptomycin} = \frac{\text{Milligrams of streptomycin} \times 100}{\text{Milligrams of dihydrostreptomycin found in the sample used}}$$

(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° and not more than +121°.

(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 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) [Reserved]

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

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 40 milligrams of gentamicin per milliliter.