

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.

(5) *Specific rotation.* Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1.0-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(6) *Content of gentamicins C₁, C_{1a}, and C₂.* Proceed as directed in § 444.20a(b)(8).

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

§ 444.20a Sterile gentamicin sulfate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Sterile gentamicin sulfate is the sulfate salt of a kind of gentamicin or a mixture of two or more such salts. It is a powder, white to buff in color. It is readily soluble in water but insoluble in ethanol. It is so purified and dried that:

(i) Its potency is not less than 590 micrograms of gentamicin per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 18.0 percent.

(vi) Its pH in an aqueous solution containing 40 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(vii) Its specific rotation in an aqueous solution containing 10 milligrams per milliliter at 25° C. is not less than +107° and not more than +121°.

(viii) Its content of gentamicin C₁ is not less than 25 nor more than 50 percent; of gentamicin C_{1a}, not less than 15 nor more than 40 percent; and of gentamicin C₂, not less than 20 nor more than 50 percent.

(ix) It gives a positive identity test for gentamicin sulfate.

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

(3) *Requests for certification; samples.* In addition to complying with the re-

quirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, specific rotation, content of gentamicins C₁, C_{1a}, and C₂, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with sufficient solution 3 to give a reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

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

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10.0 milligrams of gentamicin per milliliter.

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

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

(7) *Specific rotation.* Accurately weigh the sample to be tested in a volumetric flask and dilute with sufficient distilled water to give a solution containing approximately 10 milligrams per milliliter. Proceed as directed in § 436.210 of this chapter, using a 1-decimeter polarimeter tube and calculate the specific rotation on an anhydrous basis.

(8) *Content of gentamicins C₁, C_{1a}, and C₂—(i) Equipment—(a) Chamber (chromatographic).* Use a suitable chromatography jar with a tightly fitting, ground glass contact top for descending chromatography.

(b) *Sheets (chromatographic).* Cut a 57 × 46-centimeter sheet of Whatman No. 2