

(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

filter paper, or chromatographic paper that will produce similar results, into four strips of about 14.25 × 46 centimeters. Draw a starting line 9 centimeters from one end and mark two dots on this line, each 4 centimeters from each edge.

(ii) *Reagents.* Use reagent grade solvents and chemicals.

(iii) *Solvent system.* In each of two separators, equilibrate 200 milliliters of chloroform and 100 milliliters of methanol with 100 milliliters of 17 percent (9 molar) ammonium hydroxide. Without allowing the phases of one to separate, add the entire mixture to the chromatography jar and allow 24 hours for saturation. Allow the second separator to stand until the phases separate and use the lower phase only as the chromatographic solvent.

(iv) *Ninhydrin reagent.* To 1 gram of ninhydrin and 0.1 gram of cadmium acetate, add 3 milliliters of water and 1.5 milliliters of glacial acetic acid and shake. Add 100 milliliters of *n*-propanol and shake until solution is complete. Keep this solution in a brown bottle under refrigeration.

(v) *Procedure.* Prepare an aqueous solution containing 40 milligrams of the sample per milliliter. Apply 5 microliters of this solution to each dot on the sheet. Prepare two such sheets and place them in the tank so that elution

will take place from separate troughs. Fill the two troughs with the chromatographic solvent. Develop the sheets in a descending manner until the solvent front reaches the bottom of the paper (approximately 3½ hours at 25° C.). Remove the sheets and dry in a hood for 30 minutes. Cut each sheet in half, lengthwise. Spray one half with ninhydrin reagent and place the sprayed strip in a drying oven at 100° C. for 1 minute. The gentamicin fractions appear as reddish zones. The zone furthest from the origin is gentamicin C₁, the one closest is gentamicin C_{1a}, and the middle zone is gentamicin C₂. Cut the corresponding zones out of the other unsprayed half of the sheet. Cut each portion of the sheet thus obtained into small strips and put those from each zone into a separate 125-milliliter glass-stoppered flask. Add 50 milliliters of 0.1M potassium phosphate buffer, pH 8, to each flask and swirl the flask mechanically for 30 minutes. Decant the solution from each flask into separate test tubes and allow the paper to settle. Pipet 4 milliliters of each clear solution into a 25-milliliter volumetric flask and make to volume with the pH 8 buffer. Assay these solutions as directed in paragraph (b)(1) of this section.

(vi) *Calculations.*

$$\begin{aligned} \text{Total gentamicins} &= \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} + \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} + \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977} \\ \text{Percent of gentamicin } C_1 &= \frac{\text{Assay of } C_1 \text{ fraction}}{0.786} \times \frac{100}{\text{Total gentamicins}} \\ \text{Percent of gentamicin } C_2 &= \frac{\text{Assay of } C_2 \text{ fraction}}{1.023} \times \frac{100}{\text{Total gentamicins}} \\ \text{Percent of gentamicin } C_{1a} &= \frac{\text{Assay of } C_{1a} \text{ fraction}}{0.977} \times \frac{100}{\text{Total gentamicins}} \end{aligned}$$

where:

The assays are expressed in terms of the microgram equivalents of gentamicin; and

The factors 0.786, 1.023, and 0.977 represent

the activities of gentamicins C₁, C₂, and C_{1a} relative to the gentamicin activity of the gentamicin master standard.

(9) *Identity*. Proceed as directed in § 436.211 of this chapter, using a 0.5 percent mixture of the sample in a potassium bromide disc prepared as described in paragraph (b)(2) of that section.

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

§ 444.30 Kanamycin sulfate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Kanamycin sulfate is the sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on an anhydrous basis is not less than 750 micrograms of kanamycin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 4 percent.

(iv) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5

(v) Its residue on ignition is not more than 1.0 percent.

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

(vii) It contains not more than 5.0 percent kanamycin B.

(viii) It is crystalline.

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

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

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, identity, kanamycin B content, and crystallinity.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—(1) Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 10

micrograms of kanamycin per milliliter (estimated).

(2) [Reserved]

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

(4) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(5) *Residue on ignition*. Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity*. Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water, and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol; then add 0.5 milliliter of pyridine. Heat in a steam bath for 5 minutes and add 10 milliliters of water; a deep-purple color is produced.

(7) *Kanamycin B content—(i) Cylinders (cups)*. Use cylinders described under § 440.80a(b)(1)(i) of this chapter.

(ii) *Culture medium*. Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make agar for the base and seed layers as follows:

Peptone.....	6.0 gm.
Yeast extract.....	3.0 gm.
Beef extract.....	1.5 gm.
Agar.....	15.0 gm.
pH 7.8 to 8.0 after sterilization.	
Distilled water, q.s.....	1,000.00 ml.

(iii) *Working standard*. Dissolve a suitable quantity of the kanamycin sulfate working standard, accurately weighed, in 0.1M potassium phosphate buffer, pH 8.0, to give a concentration equivalent to 1.0 milligram of kanamycin per milliliter.

(iv) *Preparation of sample*. To 100 milligrams, accurately weighed, of kanamycin sulfate in a suitable container (such as a 7.5-milliliter serum vial) add 5.0 milliliters of 6N hydrochloric acid, and tightly close the container. Heat in a water bath at 100° C. for 1 hour and cool. Add 4 milliliters of 6N sodium hydroxide, then dilute with sterile 0.1M potassium phosphate buffer, pH 8.0, to obtain a concentration of the equivalent of 1 microgram of kanamycin per milliliter (estimated).