

Subpart A—Bulk Drugs

§ 444.6 Amikacin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amikacin is A-3-amino-3-deoxy- α -D-glucopyranosyl (1-6) - A - [6 - amino - 6 - deoxy - α - D - glucopyranosyl (1-4)] - N¹ - [(s) - 4 - amino - 2 - hydroxy - 1 - oxobutyl] - 2 - deoxy - D - streptamine. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 8.5 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 9.5 and not more than 11.5.

(v) It gives a positive identity test for amikacin.

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

(vii) Its specific rotation is not less than +97° and not more than +105°.

(viii) It is crystalline.

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

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

(i) Results of tests and assays on the batch for potency, safety, moisture, pH, identity, residue on ignition, specific rotation, and crystallinity.

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

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 10.0 micrograms of amikacin per milliliter (estimated).

(2) [Reserved]

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

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

(5) *Identity.* Proceed as directed in § 436.318 of this chapter.

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

(7) *Specific rotation.* Proceed as directed in § 436.210 of this chapter, using an aqueous solution containing 20 milligrams of amikacin per milliliter and a 1.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

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

[41 FR 49483, Nov. 9, 1976, as amended at 44 FR 10379, Feb. 20, 1979; 50 FR 19919, May 13, 1985]

§ 444.7 Amikacin sulfate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amikacin sulfate is the sulfate salt of D-streptamine, *o*-3-amino-3-deoxy- α -D-glucopyranosyl(1-6)-*o*-[6-amino-6-deoxy- α -D-glucopyranosyl(1-4)]-N¹-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-. It is so purified and dried that:

(i) Its potency is not less than 674 micrograms and not more than 786 micrograms per milligram on an anhydrous basis if the molar ratio of amikacin to sulfuric acid (H₂SO₄) is 1:2 and is not less than 691 micrograms and not more than 806 micrograms per milligram on an anhydrous basis if the molar ratio of amikacin to H₂SO₄ is 1:1.8.

(ii) Its loss on drying is not more than 13.0 percent.

(iii) The pH of an aqueous solution containing 10 milligrams of amikacin sulfate per milliliter is not less than 2.0 and not more than 4.0 if the molar ratio of amikacin to H₂SO₄ is 1:2 and not less than 6.0 and not more than 7.3 if the molar ratio of amikacin to H₂SO₄ is 1:1.8.

(iv) It gives a positive identify test for amikacin.

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

(vi) Its specific rotation is not less than +76° and not more than +84° on the anhydrous basis.

(vii) It is crystalline.

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

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

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

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.216 of this chapter, using a 25-centimeter by 4.6-millimeter column packed with irregular 5-micron octadecyl hydrocarbon bonded silica, thermostatted at 30°C, an ultraviolet detection system operating at a wavelength of 340 nanometers, a flow rate not exceeding 2.0 milliliters per minute, a chart speed of 1.0 centimeter per minute (the chart speed is increased to 5.0 centimeters per minute to obtain chromatograms used for performance parameter determinations), and a known injection volume between 15.0 and 30.0 microliters. Retention times of amikacin and kanamycin are about 10 and 15 minutes, respectively. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents—(A) 1.0 percent 2,4,6-trinitrobenzenesulphonic acid solution.* Dissolve 1.0 gram of 2,4,6-trinitrobenzenesulphonic acid in 100 milliliters of distilled water.

(B) *0.02M potassium dihydrogen phosphate.* Dissolve 2.72 grams of potassium dihydrogen phosphate in 800 milliliters of distilled water and mix to dissolve the solid. Dilute to 1,000 milliliters with distilled water and mix.

(C) *Mobile phase.* Mix 0.02M potassium dihydrogen phosphate and methanol, high performance liquid chromatography reagent grade (28:72 by volume). Adjust the pH to 6.5 with 0.4M potassium hydroxide. Filter the mobile phase through a suitable glass filter or equivalent which is capable of removing particulate matter contamination greater than 0.5 micron in diameter.

Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard and sample solutions.* (A) *Working standard solution.* Dissolve an accurately weighed portion of the amikacin working standard with sufficient distilled water to obtain a solution containing approximately 1.0 milligram of amikacin activity per milliliter. This preparation is stable for 1 week. Transfer 50 microliters of this solution directly to the bottom of a 50-milliliter, glass-stoppered centrifuge tube, using an automatic micropipetter. Add 3.2 milliliters of pyridine and 2.0 milliliters of 1 percent 2,4,6-trinitrobenzenesulphonic acid reagent just above the surface of the solution in the centrifuge tube. Close the tube tightly, mix and heat the tube in a water bath maintained at 75°C±1° for 45 minutes. Remove the tube from the bath and cool it at room temperature. Filter the contents through a 0.5 micron membrane. Use the filtrate for the quantitative chromatographic determinations.

(B) *Preparation of sample solution.* Dissolve an accurately weighed portion of sample with sufficient distilled water to obtain a solution containing 1.0 milligram of amikacin activity per milliliter (estimated). This preparation is stable for 1 week. Proceed as directed in paragraph (b)(1)(ii)(A) of this section, beginning at "Transfer 50 microliters * * *".

(C) *Resolution test solution.* Prepare an aqueous solution containing about 1.0 milligram per milliliter each of amikacin and kanamycin. Proceed as directed in paragraph (b)(1)(ii)(A) of this section, beginning at "Transfer 50 microliters * * *".

(iii) *System suitability requirements—*

(A) *Asymmetry factor.* The asymmetry factor (A_s) of the amikacin peak is satisfactory if it is not more than 1.3 at 10 percent of peak height.

(B) *Efficiency of the column.* The absolute efficiency (h_r) is satisfactory if it is not more than 20.0 for the amikacin peak.

(C) *Resolution.* The resolution (R) between the amikacin peak and the kanamycin peak is satisfactory if it is not less than 5.0.

(D) *Coefficient of variation* (relative standard deviation). The coefficient of variation (S_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