

dilution will give 25 percent light transmission and the usual inoculum for each 100 milliliters of agar for the seed layer is 0.2 milliliter of diluted suspension.

(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 § 440.80a(b)(3) of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution containing 10 milligrams of neomycin per milliliter in pyrogen-free, sterile U.S.P. saline T.S.

(4) [Reserved]

(5) *Moisture*. In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorus pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(6) *pH*. Proceed as directed in § 440.80a(b)(5)(ii) of this chapter, using a solution containing 33 milligrams of neomycin per milliliter.

(7) *Identity*—(i) *Reagents*. (a) Sulfuric acid solution: Mix concentrated sulfuric acid and distilled water in volumetric proportions of 40:60.

(b) Xylene.

(c) *p*-Bromoaniline: (Prepare and store this reagent in brown, nonactinic glassware.) Place 380 milliliters of thioureasaturated glacial acetic acid solution in the bottle, add 10 milliliters of 20 percent sodium chloride solution, 5 milliliters of 5 percent oxalic acid solution, and 5 milliliters of 10 percent disodium phosphate solution, and mix well. Add 8 grams of *p*-bromoaniline and mix well. Let this reagent stand overnight before use. Prepare the reagent once weekly.

(ii) *Procedure*. Place about 10 milligrams of the sample into a test tube (19 millimeters × 150 millimeters), dissolve with 1 milliliter of water, and then carefully add 5 milliliters of the sulfuric acid solution. Heat in a boiling water bath for 100 minutes. Cool to room temperature. Add 10 milliliters of xylene to the test tube. Stopper the tube and shake vigorously for about 1 minute. Let the two layers separate and then decant the xylene layer into a second test tube. Add 10 milliliters of the *p*-bromoaniline reagent to the xylene solution, shake, and let stand. The development of a vivid pink-red color is a positive identity test for neomycin.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985; 53 FR 12660, Apr. 15, 1988]

§ 444.46 Netilmicin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Netilmicin sulfate is the sulfate salt of D-Streptamine, 4-*O*-[3-amino-6-(aminomethyl)-3,4-dihydro-2H-pyran-2-yl]-2-deoxy-6-*O*-[3-deoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl]-*N*¹-ethyl-, (2*S*-*cis*-), (2:5). It is a white-to-buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 595 micrograms of netilmicin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 15.0 percent.

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

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

(v) Its specific rotation in an aqueous solution containing 30 milligrams per milliliter at 25° C is not less than +88° and not more than +96°.

(vi) 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, loss on drying, pH,

residue on ignition, specific rotation, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 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 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to obtain a stock solution of convenient concentration. Dilute an aliquot of the stock solution with solution 3 to the reference concentration of 0.1 microgram of netilmicin per milliliter (estimated).

(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 40 milligrams per milliliter.

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

(5) *Specific rotation*. Use an aqueous solution containing 3 milligrams of sample per milliliter. Proceed as directed in §436.210 of this chapter, using a 1.0-decimeter tube, and calculate the specific rotation on an anhydrous basis.

(6) *Identity*. Proceed as directed in §436.318 of this chapter, except:

(i) Prepare sample and standard solutions containing 10 milligrams of netilmicin per milliliter;

(ii) Use 5 microliters of the solutions to spot the chromatography plate;

(iii) Remove the plate from the tank after 1.5 hours; and

(iv) Netilmicin sulfate appears as a brown spot.

[48 FR 18800, Apr. 26, 1983; 48 FR 22144, May 17, 1983, as amended at 55 FR 11584, Mar. 29, 1990]

§444.50 Paromomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Paromomycin sulfate is the sulfate salt of a kind of paromomycin or a mixture of two or more such salts. It is a creamy-white to light-yellow powder. It is so purified and dried that:

(i) Its potency is not less than 675 micrograms per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 5.0 percent.

(iv) The pH of a 3.0 percent aqueous solution is not less than 5.0 and not more than 7.5.

(v) Its specific rotation at 25° C. in water is not less than +50° and not more than +55° on an anhydrous basis.

(vi) Its residue on ignition is not more than 2.0 percent.

(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 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, and residue on ignition.

(ii) Samples of the batch: 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 0.1M 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 1.0 microgram of paromomycin 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 3.0 percent aqueous solution.

(5) *Specific rotation*. Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter volumetric flask. Dissolve in a few milliliters of water, add water to volume, and mix. Proceed as directed in §436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

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

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