

in a glass-stoppered 50-milliliter Erlenmeyer flask and also proceed as directed in paragraph (b)(8) (vi), (vii), and (viii) of this section.

(b) *Calculation.* Calculate the recovery of total capreomycins as follows:

$$\text{Recovery of total capreomycins} = \frac{At - Ab}{A_s} \times 100$$

where:

At—Absorbance of the eluate from the unchromatographed sheet;

Ab—Absorbance of the eluate from the unchromatographed blank sheet;

As—Absorbance of the capreomycin sample solution described in paragraph (b)(8)(vii) of this section.

To be a valid assay, the recovery of total capreomycins from the unchromatographed sheet must be 100±2 percent.

(9) *Residue on ignition.* Proceed as directed in §436.207(a) of this chapter, except ignite at 700° C.

(10) *Heavy metals.* Proceed as directed in §436.208 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 448.20a Sterile colistimethate sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistimethate sodium is the sodium salt of a kind of colistin methane sulfonate or a mixture of two or more such salts. It is a white to slightly yellow, odorless, fine powder which is freely soluble in water. It is so purified and dried that:

(i) Its potency is not less than 390 micrograms of colistin base equivalent per milligram. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of colistin base equivalent that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its loss on drying is not more than 7.0 percent.

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

(vii) It gives a positive identity test for colistimethate sodium.

(viii) It passes the test for free colistin.

(ix) Its heavy metals content is not more than 30 parts per million.

(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, sterility, pyrogens, loss on drying, pH, identity, free colistin, and heavy metals.

(ii) Samples required:

(a) If the batch is packaged for repackaging or for use in the manufacture of another drug:

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

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

(b) If the batch is packaged for dispensing:

(1) For all tests except sterility: A minimum of 12 vials or if each vial contains less than 150 milligrams of colistimethate, a minimum of 60 vials.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: If the batch is packaged for repackaging or for use in manufacturing another drug, dissolve an accurately weighed sample in 2 milliliters of sterile distilled water and further dilute with sufficient 10-percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. If it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if the container is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Further dilute the

stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin base equivalent 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) *Pyrogens*. Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of colistin base equivalent per milliliter.

(4) [Reserved]

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

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a 1-percent aqueous solution prepared in the following manner: Weigh accurately 0.5 gram of sample and transfer to a 125-milliliter Erlenmeyer flask. Add 50 milliliters of freshly boiled distilled water, stopper, and shake until the sample is in solution.

(7) *Identity*—(i) *Infrared*. Proceed as directed in § 436.211 of this chapter, using a 1-percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(ii) *Iodine reduction*. Dissolve 40 milligrams of sample in 1.0 milliliter of 1.0*N* hydrochloric acid and add 0.5 milliliter of 0.02*N* iodine. The color is rapidly discharged.

(8) *Free colistin*. Dissolve 80 milligrams of sample in 3.0 milliliters of distilled water and add 0.05 milliliter of 10 percent w/v solution of silicotungstic acid. It passes the test for free colistin if no immediate precipitate is produced.

(9) *Heavy metals*. Proceed as directed in § 436.208 of this chapter.

[39 FR 19115, May 30, 1974, as amended at 44 FR 10381, Feb. 20, 1979; 44 FR 22059, Apr. 13, 1979; 50 FR 19920, May 13, 1985]

§ 448.21 Colistin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Colistin sulfate is the white to slightly yellow, odorless sulfate salt of a kind of colistin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 500 micrograms of colistin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 7.0.

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

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

(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, and identity.

(ii) Samples required on the batch: 10 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 2 milliliters of sterile distilled water and further dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 6 to the reference concentration of 1.0 microgram of colistin 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 an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity*. To about 20 milligrams of sample, add 2.0 milliliters of pH 7.0 buffer (prepared by adding 29.63 milliliters of 1 *N* sodium hydroxide to 50 milliliters of 1 *M* potassium dihydrogen phosphate, adjusting to pH 7.0 if necessary, and diluting to 100 milliliters with distilled water) and 0.2 milliliter of a 0.5 percent aqueous triketohydrindene hydrate solution, and bring to boil. A purple color is produced.

[39 FR 19115, May 30, 1974, as amended at 50 FR 19920, May 13, 1985]