

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.

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#### § 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.

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