

dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

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

(4) *Metal particles*. Proceed as directed in § 436.206 of this chapter.

[42 FR 27231, May 27, 1977, as amended at 47 FR 23443, May 28, 1982; 48 FR 21564, May 13, 1983; 50 FR 19920, May 13, 1985]

§ 448.321 Colistin sulfate for ophthalmic solution.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Colistin sulfate for ophthalmic solution is a dry mixture of colistin sulfate and mannitol packaged in combination with a suitable and harmless diluting solution which contains buffers and a preservative. When reconstituted as directed in the labeling, each milliliter contains 1.2 milligrams of colistin. Its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of colistin that it is represented to contain. It is sterile. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 6.3. The colistin sulfate used conforms to the standards prescribed by § 448.21(a)(1).

(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 the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(b) The batch for potency, sterility, loss on drying, and pH.

(ii) Samples required:

(a) The colistin sulfate used in making the batch: 10 containers, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 6 immediate containers.

(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: Reconstitute as directed in the labeling. Remove an accurately measured representative portion of the reconstituted solution and dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 1.0 microgram of colistin 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) *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 the solution reconstituted as directed in the labeling.

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

§ 448.330 Polymyxin B sulfate-trimethoprim hemisulfate ophthalmic solution.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity*. Polymyxin B sulfate-trimethoprim hemisulfate ophthalmic solution contains, in each milliliter, 10,000 units of polymyxin B and 1.0 milligram of trimethoprim in a suitable and harmless isotonic aqueous vehicle. It contains suitable and harmless buffers and preservatives. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its trimethoprim content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of trimethoprim that it is represented to contain. It is sterile. Its pH is not less than 3.0 and not more than 5.5. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1).

(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:

(A) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(B) The trimethoprim used in making the batch for all U.S.P. specifications.

(C) The batch for polymyxin B content, trimethoprim content, sterility, and pH.

(ii) Samples if required by the Director, Center for Drug Evaluation and Research:

(A) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The trimethoprim hemisulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(C) The batch:

(1) For all tests except sterility: A minimum of 7 immediate containers.

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

(b) *Tests and methods of assay*—(1) *Polymyxin content*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with 10 percent potassium phosphate buffer, pH 6.0 (solution 10), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Trimethoprim content*. Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, and a column packed with octyl, octadecyl, or phenyl groups chemically bonded to porous silica ranging from 3 to 10 micrometers in particle size. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements and calculations are as follows:

(i) *Reagents*—(A) *Diluting fluid*. 13 percent acetonitrile in 0.01M hydrochloric acid.

(B) *Mobile phase*. Mix 0.015M ethanesulfonic acid in acetonitrile; water (130:870) and adjust to pH 3.5 with 50 percent w/w sodium hydroxide and

hydrochloric acid solution. Filter the mobile phase through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution*. Place approximately 40 milligrams of trimethoprim working standard, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with 13 percent acetonitrile in 0.01M hydrochloric acid, and mix. Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, and dilute to volume with 13 percent acetonitrile in 0.01M hydrochloric acid, and mix.

(B) *Sample solution*. Transfer 4.0 milliliters of the sample into a 50-milliliter volumetric flask and dilute to volume with 13 percent acetonitrile in 0.01M hydrochloric acid.

(C) *Resolution test solution*. Place approximately 40 milligrams of trimethoprim working standard and 15 milligrams of 2-amino-4-hydroxy-5-(3',4'5')-trimethoxybenzyl pyrimidine, accurately weighed, into a 50-milliliter volumetric flask. Dissolve and dilute to volume with 13 percent acetonitrile in 0.01M hydrochloric acid, and mix. Transfer 5 milliliters of this solution to a 50-milliliter volumetric flask, and dilute to volume with 13 percent acetonitrile in 0.01M hydrochloric acid, and mix. Prepare the resolution test solution just prior to its introduction into the chromatograph pumping system.

(iii) *System suitability requirements*—(A) *Asymmetry factor*. The asymmetry factor (A_s) is satisfactory if it is not more than 1.4 at 10 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (n) is satisfactory if it is greater than 1,500 theoretical plates.

(C) *Resolution*. The resolution (R) between 2-amino-4-hydroxy-5-(3',4',5')-trimethoxybenzyl pyrimidine (AHTP) and trimethoprim is satisfactory if it is not more than 1.5.

(D) *Coefficient of variation*. The coefficient of variation (S_R 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 milligrams of trimethoprim per milliliter of sample as follows:

$$\text{Milligrams of trimethoprim per milliliter} = \frac{A_u \times W_s \times 0.8555}{A_s \times 40}$$

where:

A_u =Area of the trimethoprim peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s =Area of the trimethoprim peak in the chromatogram of the trimethoprim working standard;

W_s =Weight of the trimethoprim working standard in milligrams; and

0.855=Gravimetric conversion factor trimethoprim hemisulfate to trimethoprim.

(3) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted sample.

[54 FR 38376, Sept. 18, 1989, as amended at 59 FR 8399, Feb. 22, 1994]

Subpart E—Otic Dosage Forms

§ 448.421 Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Colistin sulfate-neomycin sulfate-thonzonium bromide-hydrocortisone acetate otic suspension is a suspension containing colistin sulfate, neomycin sulfate, thonzonium bromide, and hydrocortisone acetate, and one or more preservatives, dispersing agents, and buffer substances. Each milliliter contains 3.0 milligrams of colistin, 3.3 milligrams of neomycin, 0.5 milligram of thonzonium bromide, and 10 milligrams of hydrocortisone acetate. Its content of colistin is satisfactory if it is not less than 90 percent and not more than 135 percent of the number of milligrams of colistin per milliliter that it is represented to contain. Its content of neomycin is satisfactory

if it is not less than 90 percent and not more than 125 percent of the number of milligrams of neomycin per milliliter that it is represented to contain. It is sterile. Its pH is not less than 4.8 and not more than 5.2. The colistin sulfate used conforms to the standards prescribed therefor by §448.21(a)(1). The neomycin sulfate used conforms to the standards prescribed in §444.42a(a)(1) (i), (v), (vi), and (vii) of this chapter.

(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:

(a) The colistin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(b) The neomycin sulfate used in making the batch for potency, loss on drying, pH, and identity.

(c) The batch for colistin content, neomycin content, sterility, and pH.

(ii) Samples required:

(a) The colistin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch:

(1) For all tests except sterility: A minimum of six immediate containers.

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

(b) *Tests and methods of assay—(1) Potency—(i) Colistin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Thoroughly mix the sample and transfer an accurately measured representative portion of the sample into a 100-milliliter volumetric flask. Fill the flask to mark with 10 percent potassium phosphate buffer, pH 6.0 (solution 6). Further dilute with solution 6 to the reference concentration of 1.0 microgram of colistin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: