

(1) For all tests except sterility: A minimum of 17 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) *Bacitracin content*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100 units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the final sample solution.) Remove an aliquot and further dilute with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Polymyxin B content*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6) and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further 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 meth-

od 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 49 FR 34351, Aug. 30, 1984; 50 FR 19920, May 13, 1985]

**§ 448.313b Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ophthalmic ointment; bacitracin zinc-neomycin sulfate-polymyxin B sulfate-hydrocortisone ophthalmic ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Bacitracin zinc-neomycin sulfate-polymyxin B sulfate ophthalmic ointment is bacitracin zinc, neomycin sulfate, and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains:

(i) 400 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B with or without 10 milligrams of hydrocortisone acetate;

(ii) 500 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B; or

(iii) 400 units of bacitracin, 3.5 milligrams of neomycin, 10,000 units of polymyxin B, and 10 milligrams of hydrocortisone.

Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of bacitracin that it is represented to contain. Its neomycin content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of milligrams of neomycin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin zinc used conforms to the standards prescribed by §448.13a(a)(1). The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) of this chapter, except pyrogens. The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1), except

pyrogens, residue on ignition, and heavy metals.

(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 bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

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

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

(d) the batch for bacitracin content, neomycin content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(b) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(c) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(d) The batch:

(1) For all tests except sterility: A minimum of 17 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) *Bacitracin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. (If the bacitracin content is less than 100

units per milliliter in 0.01*N* hydrochloric acid, add sufficient additional hydrochloric acid to each concentration of the standard response line so that each standard solution contains the same amount of acid as the 1.0 unit per milliliter sample solution.) Remove an aliquot and further dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1) to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

(ii) *Neomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*M* potassium phosphate buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of 3 more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further

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: