

accurately measured portion from each container. Dilute with ethanol to obtain a stock solution of 1 milligram of cyclosporine activity per milliliter (estimated).

(ii) *Calculations.* Calculate the cyclosporine content of the vial as follows:

$$\text{Milligrams of cyclosporine per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1,000}$$

where:

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

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

P_s =Cyclosporine activity in the cyclosporine working standard solution in micrograms per milliliter; and

d =Dilution factor of the sample.

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

(3) *Bacterial endotoxins.* Proceed as directed in the United States Pharmacopeia XX bacterial endotoxins test.

[49 FR 22633, May 31, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§ 448.230 Sterile polymyxin B sulfate.

The requirements for certification and the tests and methods of assay for sterile polymyxin B sulfate packaged for dispensing are described in § 448.30a.

[44 FR 10379, Feb. 20, 1979]

Subpart D—Ophthalmic Dosage Forms

§ 448.310 Bacitracin ophthalmic dosage forms.

§ 448.310a [Reserved]

§ 448.310b Bacitracin-neomycin sulfate-polymyxin B sulfate ophthalmic ointment.

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

Each gram contains 500 units of bacitracin, 3.5 milligrams of neomycin, and 10,000 units of polymyxin B. 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 used conforms to the standards prescribed by § 448.10a(a)(1), except pyrogens, residue on ignition, and heavy metals. 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 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 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 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 assays*—(1) *Potency*—(i) *Bacitracin content*. Proceed as directed for bacitracin zinc 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 1 percent potassium phosphate buffer, pH 6.0 (solution 1), 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 1. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 1. Remove an aliquot, add sufficient hydrochloric acid so that the amount of acid in the final solution will be the same as in the reference concentration of the working standard and further dilute with 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 0.1M 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 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 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 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 27230, May 27, 1977, as amended at 47 FR 23442, May 28, 1982; 50 FR 19920, May 13, 1985]

§ 448.310c Bacitracin ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Bacitracin ophthalmic ointment contains bacitracin in a suitable and harmless ointment base. Each gram contains 500 units of bacitracin. Its potency 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. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The bacitracin used conforms to the standards prescribed by § 448.10a(a)(1), except pyrogens, residue on ignition, and heavy metals.