

that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for particulate contamination. The gentamicin sulfate used conforms to the standards prescribed therefor by § 444.20a(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 gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicins C<sub>1</sub>, C<sub>1a</sub>, and C<sub>2</sub>, and identity.

(b) The batch for gentamicin potency, sterility, moisture, and particulate contamination.

(ii) Samples required:

(a) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 15 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, except prepare the sample as follows: Place an accurately weighed representative portion of the ointment into a separatory funnel containing 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 with new portions of solution 3. Repeat any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 0.1 microgram of gentamicin 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) *Particulate contamination.* Proceed as directed in § 436.206 of this chapter.

[39 FR 19046, May 30, 1974, as amended at 50 FR 19919, May 13, 1985]

**§ 444.320c Gentamicin sulfate-prednisolone acetate ophthalmic suspension.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gentamicin sulfate-prednisolone acetate ophthalmic suspension is an aqueous suspension containing in each milliliter gentamicin sulfate equivalent to 3.0 milligrams of gentamicin and 10.0 milligrams of prednisolone acetate. It contains suitable and harmless chelating agents, tonicity agents, buffers, and preservatives. Its gentamicin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of gentamicin that it is represented to contain. Its prednisolone acetate content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of prednisolone acetate that it is represented to contain. Its pH is not less than 5.4 and not more than 6.6. It is sterile. The gentamicin sulfate used conforms to the standards prescribed by § 444.20(a)(1). The prednisolone acetate used conforms to the standards prescribed by the USP XXI.

(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 gentamicin sulfate used in making the batch for potency, loss on drying, pH, specific rotation, content of gentamicin C<sub>1</sub>, C<sub>1a</sub>, C<sub>2</sub>, and identify.

(B) The prednisolone acetate used in making the batch for all USP XXI specifications.

(C) The batch for gentamicin content, prednisolone acetate content, sterility, and pH.

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

(A) The gentamicin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(B) The batch:

(1) For all tests except sterility: A minimum of 15 immediate containers.

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

(b) *Tests and methods of assay*—(1) *Gentamicin 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 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to the reference concentration of 0.1 microgram of gentamicin per milliliter (estimated).

(2) *Prednisolone acetate content*. Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with octadecyl hydrocarbon bonded silicas, a flow rate of 2.0 milliliters per minute, and an injection volume of 30 microliters. Mobile phase, reference standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Mix acetonitrile distilled deionized water (40:60). Filter the mobile phase through a suitable glass fiber filter or equivalent which is capable of removing particulate contamination to 1 micron in diameter.

(ii) *Reference standard and sample solutions*—(A) *Preparation of reference standard solution*. Accurately weigh approximately 60 milligrams of prednisolone acetate reference standard into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol and mix well. Transfer 8 milliliters of this solution into a 50-milliliter volumetric flask, dilute to volume with 70 percent methyl alcohol, and mix well.

(B) *Preparation of sample solution*. Transfer 1.0 milliliter of the sample into a 50-milliliter volumetric flask, dilute to volume with 70 percent methyl alcohol, and mix well.

(iii) *System suitability requirements*—(A) *Tailing factor*. The tailing factor (*T*)

is satisfactory if it is not more than 1.25 at 5 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (*n*) is satisfactory if it is greater than 2,000 theoretical plates.

(C) *Coefficient of variation*. The coefficient of variation (*S<sub>r</sub>* in percent) of five replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculations*. Calculate the milligrams of prednisolone acetate per milliliter of sample as follows:

$$\text{Milligrams of prednisolone acetate} = \frac{A_u \times C_s \times d}{A_s}$$

where:

*A<sub>u</sub>*=Area of the prednisolone acetate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

*A<sub>s</sub>*=Area of the prednisolone acetate peak in the chromatogram of the prednisolone acetate reference standard;

*C<sub>s</sub>*=Concentration of prednisolone acetate in the reference standard solution in milligrams per milliliter; and

*d*=Dilution factor of the sample.

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

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

[53 FR 40725, Oct. 18, 1988, as amended at 59 FR 8398, Feb. 22, 1994]

**§444.320d Gentamicin sulfate-prednisolone acetate ophthalmic ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Gentamicin sulfate-prednisolone acetate ophthalmic ointment contains in each gram gentamicin sulfate equivalent to 3.0 milligrams of gentamicin and 6.0 milligrams of prednisolone acetate, with a suitable lubricant and preservative in a suitable and harmless white petrolatum base. Its gentamicin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of gentamicin that it is represented to contain. Its prednisolone acetate content is satisfactory if it is