

(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

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. It is sterile. Its moisture content is not more than 2.0 percent. It passes the test for metal particles. 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 United States Pharmacopeia.

(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>, C<sub>2</sub>, and identity.

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

(C) The batch for gentamicin content, prednisolone acetate content, sterility, moisture, and metal particles.

(ii) Samples, if required by the 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, 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) *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 3 to 10 micrometers in diameter, a flow rate of 2.0 milliliters per minute, and an injection volume of 30 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents—(A) 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. Degas the mobile phase just prior to its introduction into the chromatograph.

(B) *Internal standard solution.* Accurately weigh 135 milligrams ± 10 milligrams of fluorometholone acetate into a 50-milliliter volumetric flask. Dissolve and dilute to volume with methyl alcohol.

(ii) *Preparation of working standard and sample solutions—(A) Working standard solution.* Prepare the working standard solution fresh before injection by dissolving approximately 40 milligrams ± 2 milligrams of prednisolone acetate, accurately weighed, into a 100-milliliter volumetric flask with 25 milliliters of methyl alcohol. Sonicate to dissolve and dilute to volume with methyl alcohol and mix well. Transfer 8 milliliters of this solution into a 50-milliliter volumetric flask. Add 25 milliliters of hexane and shake. Add 2.0 milliliters of internal standard as described in paragraph (b)(2)(i)(B) of this section, and dilute to volume with methyl alcohol. Shake vigorously for 30 seconds, allow the phases to separate, then aspirate the upper hexane layer and dilute to volume with methyl alcohol. Centrifuge for 10 minutes at 5,700 revolutions per minute.

(B) *Sample solution.* Accurately weigh 500 milligrams ± 20 milligrams of the sample into a 50-milliliter volumetric

flask. Add 25 milliliters of hexane and sonicate. Add 2.0 milliliters of the internal standard. Dilute to volume with methyl alcohol. Shake vigorously for 30 seconds and allow the phase to separate. Aspirate the upper hexane and cloudy layers. Dilute to volume with methyl alcohol. Centrifuge for 10 minutes at 5,700 revolutions per minute.

(iii) *System suitability requirements—*(A) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.50 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 2,500 theoretical plates.

(C) *Resolution.* The resolution (*R*) between the peak for prednisolone acetate and the internal standard is satisfactory if it is not less than 2.0.

(D) *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. Alternate chromatographic conditions are acceptable provided comparable system suitability requirements are met. However, the sample preparation described in paragraph (b)(2)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the percent of prednisolone acetate as follows:

$$\text{Percent of prednisolone acetate (w/w)} = \frac{R_u \times P_s \times d \times 100}{R_s \times W_u}$$

where:

*R<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)/Area of internal standard peak;

*R<sub>s</sub>*=Area of the prednisolone acetate peak in the chromatogram of the prednisolone acetate working standard /Area of internal standard peak;

*P<sub>s</sub>*=Prednisolone acetate activity in the prednisolone acetate working standard solution in milligrams per milliliter;

*W<sub>u</sub>*=Weight of sample in milligrams; and

*d*=Dilution factor of the sample.

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

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

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

[55 FR 2643, Jan. 26, 1990]

**§ 444.342 Neomycin sulfate ophthalmic dosage forms.**

**§ 444.342a Neomycin sulfate-ophthalmic suspension; neomycin sulfate-ophthalmic solution (the blanks being filled in with the established name(s) of the other active ingredient(s) present in accordance with paragraph (a)(1) of this section).**

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* The drug is a suspension or a solution containing, in each milliliter, 3.5 milligrams of neomycin and the following other active ingredients in a suitable and harmless vehicle:

- (i) 15 milligrams of cortisone acetate; or
- (ii) 5 milligrams or 25 milligrams of hydrocortisone acetate; or
- (iii) 1 milligram or 2 milligrams of prednisolone; or
- (iv) 1 milligram of sodium dexamethasone phosphate; or
- (v) 5 milligrams of prednisolone phosphate.

It contains suitable and harmless buffers, dispersants, and preservatives. It is sterile. Its pH is not less than 6.0 and not more than 8.0. The neomycin sulfate used conforms to the standards prescribed by §444.42a(a)(1) (i), (vi), and (vii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter. Its expiration date is 12 months.

(3) *Request 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 neomycin sulfate used in making the batch for potency, pH, and identity.
  - (b) The batch for potency, sterility, and pH.
- (ii) Samples required:
  - (a) The neomycin sulfate used in making the batch: 10 containers, each