

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 dexamethasone content as follows:

$$\frac{\text{Milligrams of dexamethasone}}{\text{per gram}} = \frac{A_u \times P_s \times d}{A_s \times 1,000 \times n}$$

where:

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

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

P_s =Dexamethasone content in the dexamethasone working standard solution in micrograms per milliliter;

d =Dilution factor of the sample; and

n =Number of grams of sample assayed.

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

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

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

(6) *Tobramycin identity.* Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows: Weigh approximately 1 gram of the sample into a test tube. Add 1 to 2 milliliters of chloroform to the test tube and shake vigorously to dissolve the ointment. Centrifuge for approximately 15 minutes to clearly separate the layers. Use the top (aqueous) layer in the procedure.

NOTE: If an oily film remains on the top of the aqueous layer and interferes with sampling, the aqueous layer may be transferred to another test tube and washed with an additional 1 to 2 milliliters of chloroform.

(7) *Dexamethasone identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the dexamethasone working standard.

[55 FR 617, Jan. 8, 1990]

§ 444.380e Tobramycin-fluorometholone acetate ophthalmic suspension.

(a) *Requirements for certification—(1) Standards of identity, strength, quality,*

and purity. Tobramycin-fluorometholone acetate ophthalmic suspension is an aqueous suspension containing, in each milliliter, 3.0 milligrams of tobramycin and 1.0 milligram of fluorometholone acetate in a suitable and harmless aqueous vehicle. It contains one or more suitable and harmless dispersants, preservatives, buffers, and tonicity agents. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its fluorometholone acetate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of fluorometholone acetate than it is represented to contain. It is sterile. Its pH is not less than 6.0 and not more than 7.0. It passes the identity tests for tobramycin and fluorometholone acetate. The tobramycin used conforms to the standards prescribed by § 440.80(a)(1) of this chapter, except heavy metals. The fluorometholone acetate used conforms to the standards prescribed in the U.S. Pharmacopeia XXII.

(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 tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The fluorometholone acetate used in making the batch for all requirements in U.S. Pharmacopeia XXII.

(C) The batch for tobramycin potency, fluorometholone acetate content, sterility, pH, tobramycin identity, and fluorometholone acetate identity.

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

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

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

(b) *Tests and methods of assay*—(1) *Tobramycin potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Fluorometholone 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 or cartridge packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate not to exceed 2.0 milliliters per minute, and an injection volume of 10 or 20 microliters. Mobile phase, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase*. Mix acetonitrile:water (50:50) and adjust if necessary by reducing the amount of acetonitrile to increase retention, or by increasing the amount of acetonitrile to decrease the retention of the solute. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions, and resolution test solution*—(A) *Working standard solution*. Accurately weigh approximately 25 milligrams of the fluorometholone acetate working standard into a 10-milliliter volumetric flask and add about 5 milliliters of acetonitrile. Shake until dissolved. Dilute to volume with acetonitrile. Further dilute 1.0 milliliter of this solution in a volumetric flask to 10 milliliters with acetonitrile to obtain a solution of known concentration con-

taining approximately 250 micrograms of fluorometholone acetate per milliliter. Mix well.

(B) *Sample solution*. Shake vial thoroughly, to homogenize its contents, and immediately remove an accurately measured representative portion from it. Quantitatively dilute the suspension thus obtained with sufficient acetonitrile to obtain a solution containing 250 micrograms of fluorometholone acetate per milliliter (estimated). For instance, dilute a 1.0 milliliter aliquot of suspension with 3.0 milliliters of acetonitrile and filter.

(C) *Resolution test solution*. Prepare as directed in paragraph (b)(2)(ii)(A) of this section, except use 10 milligrams of fluorometholone in addition to the 25 milligrams of fluorometholone acetate working standard.

(iii) *System suitability requirements*—(A) *Asymmetry*. The asymmetry (A_s) is satisfactory if it is not more than 1.35 at 10 percent of peak height.

(B) *Efficiency of the column*. The efficiency of the column (h_p) is satisfactory if it is not greater than 20, equivalent to 1,000 plates for a 10-centimeter column of 5 microns or 2,500 plates for a 25-centimeter column of 5 micron size particles.

(C) *Resolution*. The resolution (R_s) between the peaks of fluorometholone acetate and fluorometholone is satisfactory if it is not less than 2.0.

(D) *Capacity factor*. The capacity factor (k) for fluorometholone acetate is satisfactory if it is in the range between 1.0 and 5.0.

(E) *Coefficient of variation*. The coefficient of variation (RSD in percent) of 5 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 fluorometholone acetate content of the container as follows:

$$\frac{\text{Milligrams of fluorometholone acetate per container}}{\text{acetate}} = \frac{A_U \times P_s \times d}{A_s \times 1,000}$$

where:

A_U = Area of the fluorometholone acetate peak in the chromatogram of the sample

(at a retention time equal to that observed for the standard);
A_s=Area of the fluorometholone acetate peak in the chromatogram of the fluorometholone acetate working standard;
P_s=Fluorometholone acetate content in the fluorometholone acetate working standard solution in micrograms 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 § 436.20(e)(1).

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

(5) *Tobramycin identity*. Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows: Decant 1.0 milliliter of the unshaken sample into a test tube. Add 100 milligrams of sodium sulfate to the test tube and shake until the sodium sulfate has been dispersed. Centrifuge to obtain a clear supernatant. Use the supernatant as the sample solution.

(6) *Fluorometholone acetate identity*. The high performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the fluorometholone acetate working standard.

[58 FR 26671, May 4, 1993]

Subpart E—Otic Dosage Forms

§ 444.442 Neomycin sulfate otic dosage forms.

§§ 444.442a—444.442c [Reserved]

§ 444.442d Neomycin sulfate ointment; neomycin sulfate- _____ ointment (the blank being filled in with the established name(s) of certain other active ingredient(s)).

The requirements for certification and the tests and methods of assay for neomycin sulfate ointment and for neomycin sulfate- _____ ointment are described in § 444.542a.

§ 444.442e [Reserved]

§ 444.442f Neomycin sulfate-hydrocortisone-acetic acid otic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Neomycin sulfate-hydrocortisone-acetic acid otic suspension is an aqueous suspension containing in each milliliter 5.0 milligrams of neomycin sulfate equivalent to 3.5 milligrams of neomycin and 10 milligrams of hydrocortisone. It also contains 2 percent acetic acid. It may contain one or more suitable and harmless buffers, preservatives, and dispersants. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of neomycin that it is represented to contain. It is sterile. Its pH is not less than 4.5 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed in § 444.42a(a)(1)(i), (v), (vi), and (vii).

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

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

(ii) Samples required:

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

(b) The batch:

(1) For all tests except sterility: A minimum of 5 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: Remove an accurately measured representative portion of the sample and dilute with sufficient 0.1M potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin 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, except if the steroid prevents solubilization, use 0.25 milliliter in lieu