

standards prescribed by § 446.67. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions for lay use of the drug.

(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 oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

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

(c) The batch for oxytetracycline content, polymyxin content, and loss on drying.

(ii) Samples required:

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

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar with sufficient 0.1N hydrochloric acid to ob-

tain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh 1 gram of the powder and place into a 50-milliliter centrifuge tube. Add 15 milliliters of acetone and 1 drop of concentrated hydrochloric acid and stir well. Add 20 milliliters of acetone and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant liquid and repeat the acetone-acid extraction once more. Dissolve and dilute the residue with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[43 FR 11173, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.581 Tetracycline hydrochloride dermatologic dosage forms.

§§ 446.581a–446.581b [Reserved]

§ 446.581c Tetracycline hydrochloride for topical solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for topical solution is a dry mixture of tetracycline hydrochloride, 4-epitetracycline hydrochloride, and sodium bisulfite packaged in combination with a suitable and harmless aqueous vehicle. When reconstituted as directed in the labeling, each milliliter contains 2.2 milligrams of tetracycline hydrochloride. The tetracycline hydrochloride content of the reconstituted solution is satisfactory if it contains not less than 90 percent and not more

than 130 percent of the number of milligrams of tetracycline hydrochloride per milliliter that it is represented to contain. The 4-epitetracycline hydrochloride content is satisfactory if it contains not less than 135 percent and not more than 165 percent of the amount of tetracycline hydrochloride in the reconstituted solution at the time of reconstitution. The loss on drying of the dry mixture is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.9 and not more than 3.5. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a, except sterility, pyrogens, and histamine. The 4-epitetracycline hydrochloride used conforms to the following standards: It gives a positive identity test for 4-epitetracycline hydrochloride; its 4-epitetracycline content is not less than 70 percent; its total anhydrotetracycline and 4-epianhydrotetracycline content is not more than 2.0 percent; its loss on drying is not more than 6.0 percent; its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 4.0.

(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 tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, and crystallinity.

(b) The 4-epitetracycline hydrochloride used in making the batch for 4-epitetracycline content and identity, total anhydrotetracycline and 4-epianhydrotetracycline content, loss on drying, and pH.

(c) The batch for tetracycline hydrochloride content, 4-epitetracycline hydrochloride content, loss on drying, and pH.

(ii) Samples required:

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

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay of the tetracycline hydrochloride for topical solution—(1) Tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.* Proceed as directed in § 436.340 of this chapter.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, except use the contents of one immediate container.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when reconstituted as directed in the labeling.

(c) *Tests and methods of assay of the 4-epitetracycline hydrochloride used in making the batch—(1) 4-epitetracycline content and identity.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of § 446.581c(b)(1)(iv) prepare the sample by weighing accurately 20 milligrams \pm 5 milligrams of 4-epitetracycline hydrochloride bulk powder and transfer to a 25-milliliter volumetric flask. Dissolve with 1.0 milliliter of methyl alcohol and dilute to volume with the buffer solution. Pipet a 2.0-milliliter aliquot to a 10-milliliter volumetric flask and dilute to volume with the buffer solution. Place the column in a suitable support. Place a 100-milliliter graduate under the column. Open the column stopcock, pipet 2.0 milliliters of solution from the 10-milliliter volumetric flask onto the column packing and allow the sample to permeate the column packing. Place a solvent reservoir containing 20 milliliters of benzene on top of the column and begin to collect the eluate (at flow rate of approximately 1 milliliter per minute). When the benzene level reaches the top of the column packing, replace the empty solvent reservoir with a second solvent reservoir containing 60 milliliters of chloroform and continue elution. When the chloroform level reaches the top of the column packing, replace second empty solvent reservoir with a third solvent reservoir containing 50-milliliters of the *n*-butanol:chloroform mixture and replace the 100-milliliter graduate with a 10-milliliter graduate. Collect 8.0 milliliters of eluate. Replace the 10-milliliter graduate with a 50-milliliter low-actinic volumetric flask and continue collecting the eluate containing the 4-

epitetracycline fraction until the column runs dry. Determine the absorbance of the 4-epitetracycline eluate as described in paragraph (b)(1)(v) of this section.

Calculate the 4-epitetracycline hydrochloride content of the 4-epitetracycline hydrochloride bulk powder as follows:

$$\text{Percent by weight 4-epitetracycline content} = \frac{A \times 25 \times 10 \times 50 \times 100}{a \times W \times 2 \times 2}$$

where:

A=Absorbance at 366 nanometers of the low-actinic 50-milliliter volumetric flask.

a=Previously established mean absorptivity of the tetracycline hydrochloride working standard eluates in liters/gram/centimeter with the calculation corrected for potency.

W=Weight of 4-epitetracycline hydrochloride bulk powder in milligrams.

The identity of the 4-epitetracycline hydrochloride is confirmed if the absorbance of the sample after column elution is such that the 4-epitetracycline hydrochloride content is greater than 70 percent by weight.

(2) *Total anhydrotetracycline and 4-epianhydrotetracycline content.* Proceed as directed in § 436.309 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

[42 FR 59066, Nov. 15, 1977; 43 FR 3705, Jan. 27, 1978, as amended at 48 FR 51291, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.581d Tetracycline hydrochloride ointment.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ointment contains tetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 30 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its moisture content is not more than 1 percent. The tetracycline hydrochloride used conforms to the

standards prescribed by § 446.81(a)(1), except 4-epianhydrotetracycline content.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or inserted within the package: Adequate directions under which the layperson can use the drug safely and efficaciously.

(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 tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

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

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.106 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 0.1N 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 0.1N hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and fill to volume with 0.1N hydrochloric acid to obtain a stock solution of convenient concentration containing not less than