

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 containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. 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) *Sterility*. Proceed as directed in § 436.20(e)(1) of this chapter, except dissolve the ointment as follows: Aseptically transfer a portion of 0.25 gram from each of 10 immediate containers of the drug to 400 milliliters of diluting fluid D in an Erlenmeyer flask. Repeat the procedure on another 10 immediate containers. Swirl the flasks to dissolve the ointment.

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

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

## PART 449—ANTIFUNGAL ANTIBIOTIC DRUGS

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### Subpart A—Bulk Drugs

## §§ 449.1—449.3 [Reserved]

### § 449.4 Amphotericin B.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

- (ii) It contains not more than 15 percent of amphotericin A.
- (iii) [Reserved]
- (iv) Its loss on drying is not more than 5.0 percent.
- (v) It contains not more than 3.0 percent residue on ignition.
- (vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chapter, each package shall bear on its label the statements “Store below 10° C.” and “Protect from light and moisture”.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on the batch for potency, amphotericin A content, loss on drying, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute an aliquot with dimethylsulfoxide to a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Remove an aliquot; dilute with 0.2M potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content*—(i) *Amphotericin A.* Dry approximately 20 milligrams of the nystatin working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 200-milliliter volumetric flask. Add exactly 40.0 mil-

liliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(ii) *Amphotericin B.* Dry approximately 50 milligrams of the amphotericin B working standard as described in § 436.200(a) of this chapter. Accurately weigh the dried working standard and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

The standard solution should be used for 1 day only.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested and quantitatively transfer into a 50-milliliter volumetric flask. Add 10 milliliters of dimethylsulfoxide and dissolve. Make to mark with methyl alcohol and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark and mix thoroughly.

(iv) *Blank.* Pipette 10 milliliters of dimethylsulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of nystatin standard, amphotericin B standard, and the sample at 304 nanometers and at 282 nanometers. Calculate the absorptivity of each standard at both wavelengths:

$$\text{Percent amphotericin A} = \frac{[(B \times S_2) - (b \times S_1)] \times 625}{W_s \times [(B \times a) - (b \times A)]}$$

where:

A=Absorptivity of nystatin standard at 282 nanometers;

$B$ =Absorptivity of amphotericin B standard at 282 nanometers;

$a$ =Absorptivity of nystatin standard at 304 nanometers;

$b$ =Absorptivity of amphotericin B standard at 304 nanometers;

$S_1$ =Absorbance of sample at 282 nanometers;

$S_2$ =Absorbance of sample at 304 nanometers;

$W_s$ =Weight of sample in grams (on an anhydrous basis).

(3) [Reserved]

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

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Using the solutions prepared as described in paragraphs (b)(2)(ii), (iii), and (iv) of this section, record the absorption spectrum from 320 to 240 nanometers. Then dilute these solutions (1+9) with methyl alcohol and record the absorption spectrum from 400 to 320 nanometers. The sample exhibits absorption peaks at identical wavelengths with that of the amphotericin B standard. Depending on the amphotericin A content of the sample, a peak may occur at 304 nanometers.

[39 FR 19115, May 30, 1974, as amended at 46 FR 16684, Mar. 13, 1981; 49 FR 2242, Jan. 19, 1984; 50 FR 19920, May 13, 1985]

#### § 449.4a Amphotericin B for use in parenteral products.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethylformamide and dimethylsulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms of amphotericin B per milligram on an anhydrous basis.

(ii) It contains not more than 5 percent of amphotericin A.

(iii) [Reserved]

(iv) Its loss on drying is not more than 5.0 percent.

(v) It contains not more than 0.5 percent residue on ignition.

(vi) It passes the identity test.

(2) *Labeling.* In addition to the labeling prescribed by § 432.5(b) of this chap-

ter, each package shall bear on its label the statements "Store below 10° C." and "Protect from light and moisture".

(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 the batch for potency, amphotericin A content, loss on drying, residue on ignition, and identity.

(ii) Samples required on the batch: 10 packages, each containing not less than 500 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient dimethylsulfoxide to give a stock solution of convenient concentration. Further dilute with dimethylsulfoxide to give a concentration of 20 micrograms of amphotericin B per milliliter (estimated). Dilute an aliquot with 0.2M potassium phosphate buffer, pH 10.5 (solution 10), to the reference concentration of 1.0 microgram of amphotericin B per milliliter (estimated).

(2) *Amphotericin A content.* Proceed as directed in § 449.4(b)(2).

(3) [Reserved]

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

(5) *Residue of ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Identity.* Proceed as directed in § 449.4(b)(7).

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#### § 449.10 Candicidin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Candicidin is a brown to yellow powder. It is sparingly soluble in water; very slightly soluble in ethyl alcohol, butyl alcohol, and acetone. It is so purified and dried that:

(i) Its potency is not less than 1,000 micrograms of candicidin per milligram on an anhydrous basis.

(ii) Its loss on drying is not more than 4 percent.

(iii) Its pH is not less than 8.0 nor more than 10.0 in a 1 percent aqueous suspension.