Amphotericin B

\[ \text{C}_{47} \text{H}_{73} \text{NO}_{17} \quad 924.08 \]

Amphotericin B.

\( \text{[1R,1R',3S',5R',6R',9R',11R',15S',16R',17R',18S',19E,} \)

\( \text{21E,23E,25E,27E,29E,31E,33R',35S',36R',37S']-33 \)-

\( \text{[(3-Amino-9,6-dideoxy-\text{β}-D-\text{mannopyranosyl} \text{oxy})]-1,3,5,6,9,11,17,19,21,23,25,27,29,31-}

\( \text{heptaene-36-carboxylic acid} \quad [1397-89-3]. \]

Amphotericin B has a potency of not less than 750 \( \mu \text{g} \) of \( \text{C}_{47} \text{H}_{73} \text{NO}_{17} \) per mg, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers, and store in a cool place.

Labeling—Label it to state whether it is intended for use in preparing dermatological and oral dosage forms or parenteral dosage forms.

USP Reference standards (11)—

USP Amphotericin B RS

USP Nystatin RS

Identification, Ultraviolet Absorption (197U)—

Spectral range 1: 240 to 320 nm.

Solution 1: prepared as directed for Test preparation in the Limit of amphotericin A, and compare its absorbance to that of the Amphotericin B standard preparation. An extra peak may occur at 304 nm in the spectrum of this solution.

Spectral range 2: 320 to 400 nm.

Solution 2: prepared as directed for Test preparation in the Limit of amphotericin A and then diluted with 9 volumes of methanol. Compare its absorbance to that of a similar dilution of the Amphotericin B standard preparation.

Loss on drying (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° C for 3 hours; it loses not more than 5.0% of its weight.

Residue on ignition (281): not more than 0.5%, the charred residue being moistened with 2 ml of nitric acid and 5 drops of sulfuric acid. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, yields not more than 3.0%.

Limit of amphotericin A—

Test preparation—Dissolve about 50 mg of Amphotericin B, accurately weighed, in 10.0 ml of dimethyl sulfoxide in a 50-

ml volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 ml of this solution to a 50-ml volumetric flask, dilute with methanol to volume, and mix.

Nystatin standard preparation—Dissolve about 20 mg of USP Nystatin RS, accurately weighed, in 40.0 ml of dimethyl sulfoxide in a 200-ml volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 ml of this solution to a 50-ml volumetric flask, dilute with methanol to volume, and mix.

Amphotericin B standard preparation—Dissolve about 50 mg of USP Amphotericin B RS, accurately weighed, in 10.0 ml of dimethyl sulfoxide in a 50-ml volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 ml of this solution to a 50-ml volumetric flask, dilute with methanol to volume, and mix. Prepare this solution fresh daily.

Procedure—Concomitantly determine the absorbances of the Nystatin and Amphotericin B standard preparations and the Test preparation in 1-cm cells at 304 nm and at 282 nm, with a suitable spectrophotometer, using a 1 in 62.5 solution of dimethyl sulfoxide in methanol as the blank. Calculate the percent of amphotericin A taken by the formula:

\[
25W_5\left\{\left[A_{B304} \times A_{U_{304}}\right] - \left[A_{B304} \times A_{U_{282}}\right]\right\} / \left[\left[A_{B304} \times A_{U_{282}}\right] - \left[A_{B304} \times A_{U_{304}}\right]\right]\]

in which \( W_5 \) is the weight, in mg, of USP Nystatin RS taken, \( A_{B304} \) and \( A_{B304} \) are the absorbances of the Amphotericin B standard preparation at 282 nm and 304 nm, respectively, \( A_{U_{304}} \) and \( A_{U_{282}} \) are the absorbances of the Nystatin standard preparation at 282 nm and 304 nm, respectively, \( A_{B304} \) and \( A_{B304} \) are the absorbances of the Test preparation at 282 nm and 304 nm, respectively, and \( W_5 \) is the weight, in mg, of the Amphotericin B taken: not more than 5%, calculated on the dried basis, is found. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, contains not more than 15% of amphotericin A, calculated on the dried basis.]

Assay—Proceed with amphotericin B as directed under Antibiotics—Microbial Assays (81).

Amphotericin B Cream

Amphotericin B Cream contains not less than 90.0 percent and not more than 125.0 per cent of the labeled amount of Amphotericin B.

Packaging and storage—Preserve in collapsible tubes, or other well-closed containers.

USP Reference standards (11)—

USP Amphotericin B RS

Minimum fill (755): meets the requirements.

Assay—Proceed as directed for amphotericin B under Antibiotics—Microbial Assays (81), blending a suitable accurately weighed portion of Cream in a high-speed blender with a sufficiently accurately measured volume of dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 \( \mu \text{g} \) of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with Buffer No. 10 to obtain a Test Dilution having a concentration assumed to be equal to the median dose level of the Standard.
**Amphotericin B for Injection**

Amphotericin B for Injection is a sterile complex of Amphotericin B and deoxycholate sodium and one or more suitable buffers. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of \(\text{C}_{47}\text{H}_{73}\text{NO}_{17}\).  

Packaging and storage—Preserve in Containers for Sterile Solids as described under Injections (1), in a refrigerator and protected from light.  

Labeling—Label it to indicate that it is intended for use by intravenous infusion to hospitalized patients only, and that the solution should be protected from light during administration.

**USP Reference standards** (11)—  
USP Amphotericin B RS  
USP Endotoxin RS

**Bacterial endotoxins** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of amphotericin B. For products used or labeled for intrathecal injection, it contains not more than 0.9 USP Endotoxin Unit per mg of amphotericin B.

**Sterility** (71)—It meets the requirements when tested as directed in the section Membrane Filtration under Test for Sterility of the Product to be Examined, 50 mg from each container being tested.

**pH** (791): between 7.2 and 8.0, in an aqueous solution containing 10 mg of amphotericin B per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary stopped bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

**Other requirements**—It meets the requirements for Uniformity of Dosage Units (905) and for Labeling under Injections (1).

**Assay**—  
**Assay preparation 1** (where it is packaged as a single-dose container)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

**Assay preparation 2** (where the labeling states the quantity of amphotericin B in a given volume of constituted solution)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw an accurately measured volume of the resultant solution, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

**Procedure**—Proceed as directed for amphotericin B under Antibiotics—Microbial Assays (81), using an accurately measured volume of Assay preparation diluted quantitatively and stepwise with Buffer No. 10 to obtain a Test Dilution having a concentration assumed to be equal to the median dose level of the Standard.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 5.0 and 7.0.

**Assay**—Proceed as directed for amphotericin B under Antibiotics—Microbial Assays (81), quantitatively dissolving a suitable accurately measured volume of Lotion in sufficient dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with Buffer No. 10 to obtain a Test Dilution having a concentration assumed to be equal to the median dose level of the Standard.

**Amphotericin B Ointment**

Amphotericin B Ointment is Amphotericin B in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

Packaging and storage—Preserve in collapsible tubes, or other well-closed containers.

**USP Reference standards** (11)—  
USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

**Water, Method I** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed for amphotericin B under Antibiotics—Microbial Assays (81), using an accurately weighed portion of Ointment, equivalent to about 30 mg of amphotericin B, mixed with 10.0 mL of ether in a suitable glass-stoppered conical flask and allowed to stand, with intermittent shaking, for 1 hour. Add 20.0 mL of dimethyl sulfoxide and shake by mechanical means for 10 minutes. Dilute quantitatively and stepwise with dimethyl sulfoxide to a concentration of approximately 20 µg per mL. Quantitatively dilute an accurately measured volume of this stock solution with Buffer No. 10 to obtain a Test Dilution having a concentration assumed to be equal to the median dose level of the Standard.

**Amphicillin**

\[\text{C}_{64}\text{H}_{63}\text{N}_{10}\text{O}_{5} (\text{anhydrous})\]

4-Thia-1-acabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetamido)-3,3-dimethyl-7-oxo-\(\{2,5,8,11\}\)-6-\(\{\beta\}\)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-acabicyclo[3.2.0]heptane-2-carboxylic acid [69-53-4].

Trihydrate [7177-48-2].

** DEFINITION**

Amphicillin is anhydrous or contains three molecules of water of hydration. It contains NLT 900 µg and NMT 1050 µg of \(\text{C}_{64}\text{H}_{63}\text{N}_{10}\text{O}_{5}\) per mg, calculated on the anhydrous basis.