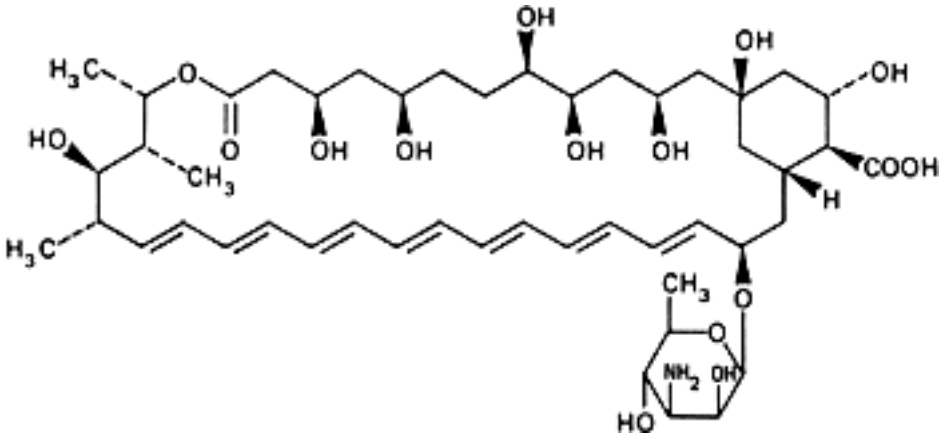
Amphotericin B (Amphotericinum B)

Amphotericin B for parenteral use

Molecular formula. $C_{47}H_{73}NO_{17}$

Relative molecular mass. 924.1

Graphic formula.



Chemical name. (1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(3-Amino-3,6-dideoxy-*β*-D-mannopyranosyl)-oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxa-bicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid; [1*R*-(1*R**,3*S**,5*R**,6*R**,9*R**,11*R**,15*S**,16*R**,17*R**,18*S**,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R**,35*S**,36*R**,37*S**)]-33-[(3-amino-3,6-dideoxy-*β*-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid; (3*R*,5*R*,8*R*,9*R*,11*S*,13*R*,15*S*,16*R*,17*S*,19*R*,34*S*,35*R*,36*R*,37*S*)-19-(3-amino-3,6-dideoxy-*β*-D-mannopyranosyloxy)-16-carboxy-3,5,8,9,11,13,15,35-octahydroxy-34,36-dimethyl-13,17-epoxyoctatriaconta-20,22,24,26,28,30,32-heptaen-37-olide; CAS Reg. No. 1397-89-3.

Description. A yellow to orange powder; odourless or almost odourless.

Solubility. Practically insoluble in water, ethanol (~750 g/l) TS, toluene R and ether R; soluble in 200 parts of dimethylformamide R and in 20 parts of dimethyl sulfoxide R, slightly soluble in methanol R.

Category. Antifungal drug.

Storage. Amphotericin B should be kept in a tightly closed container, protected from light, and stored at a temperature between 2 and 8 °C.

Labelling. The designation Amphotericin B for parenteral use indicates that the substance complies with the altered and additional requirements for Amphotericin B and may be used for parenteral administration.

Additional information. Even in the absence of light, Amphotericin B is gradually degraded on exposure to a humid atmosphere, the decomposition being faster at higher temperatures. In diluted solutions it is sensitive to light and is inactivated at low pH values.

Requirements

Definition. Amphotericin B contains not less than 750 µg per mg, calculated with reference to the dried substance.

Identity tests

A. Dissolve 25 mg in 5 mL of dimethyl sulfoxide R, add sufficient methanol R to produce 50 mL, and dilute 2.0 mL to 200 mL with methanol R. The absorption spectrum of the resulting solution, when observed between 300 nm and 450 nm, exhibits 3 maxima at about 362 nm, 381 nm, and 405 nm. The ratio of the absorbance of a 1-cm layer at 362 nm to that at 381 nm is about 0.6; the ratio of the absorbance at 381 nm to that at 405 nm is about 0.9.

B. Dissolve about 1 mg in 2.0 mL of dimethyl sulfoxide R and introduce 5 mL of phosphoric acid (~1440 g/l) TS to form a lower layer; a blue ring is immediately formed at the interface of the two liquids. Mix the two liquids; a strong blue colour is produced. Add 15 mL of water and mix; the colour of the solution changes to pale yellow.

Sulfated ash. Not more than 30 mg/g.

Loss on drying. Dry to constant weight at 60 °C under reduced pressure (not exceeding 0.6 kPa or about 5 mm of mercury); it

loses not more than 50 mg/g.

Content of tetraenes. Dissolve 0.05 g, accurately weighed, in 5 mL of dimethyl sulfoxide R, and add sufficient methanol R to produce 50 mL; dilute 4 mL to 50 mL with methanol R (solution A). For the reference solutions dissolve similarly 0.05 g, accurately weighed, of amphotericin B RS instead of the substance being examined (solution B). Further prepare a solution of 25 mg of nystatin RS, accurately weighed, in 25 mL of dimethyl sulfoxide R, and add sufficient methanol R to produce 250 mL; dilute 4 mL to 50 mL with methanol R (solution C). Measure the absorbances of a 1-cm layer of solutions A, B, and C at the maxima at about 282 nm and 304 nm, using as a blank a solution of 5 mL of dimethyl sulfoxide R diluted to 50 mL with methanol R, 4 mL of which are diluted once again to 50 mL with methanol R.

Calculate the $A^{1\%}_{1cm}$ of solutions A, B, and C at both wavelengths and then apply the following formula: $F+1000(B_1A_2-B_2A_1)/(C_2B_1-C_1B_2)$, where A_1 and A_2 are the $A^{1\%}_{1cm}$ of the substance being examined at 282 nm and 304 nm, respectively, B_1 and B_2 are the $A^{1\%}_{1cm}$ of amphotericin B RS at 282 nm and 304 nm, respectively, C_1 and C_2 are the $A^{1\%}_{1cm}$ of nystatin RS at 282 nm and 304 nm, respectively, and F is the declared content of tetraenes in amphotericin B RS; the content of tetraenes in the substance examined is not more than 150 mg/g.

Assay. Triturate 0.060 g with dimethylformamide R and add, with shaking, sufficient dimethylformamide R to produce 100 mL. Dilute 10 mL to 100 mL with dimethylformamide R and carry out the assay as described under 3.1 Microbiological assay of antibiotics, using *Saccharomyces cerevisiae* (NCTC 10716, or ATCC 9763) as the test organism, culture medium Cm3 with a final pH of 6.1, sterile phosphate buffer pH 10.5, TS1, an appropriate concentration of amphotericin B (usually between 0.5 and 10.0 µg/mL), and an incubation temperature of 29-33 °C. The precision of the assay is such that the fiducial limits of error of the estimated potency (P = 0.95) are not less than 95% and not more than 105% of the estimated potency. The upper fiducial limit of error of the estimated potency (P = 0.95) is not less than 750 µg per mg, calculated with reference to the dried substance.

Additional requirements for Amphotericin B for parenteral use

Complies with the monograph for "Parenteral preparations".

Sulfated ash. Not more than 5.0 mg/g.

Content of tetraenes. Not more than 100 mg/g.

Bacterial endotoxins. Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.9 IU of endotoxin RS per mg.