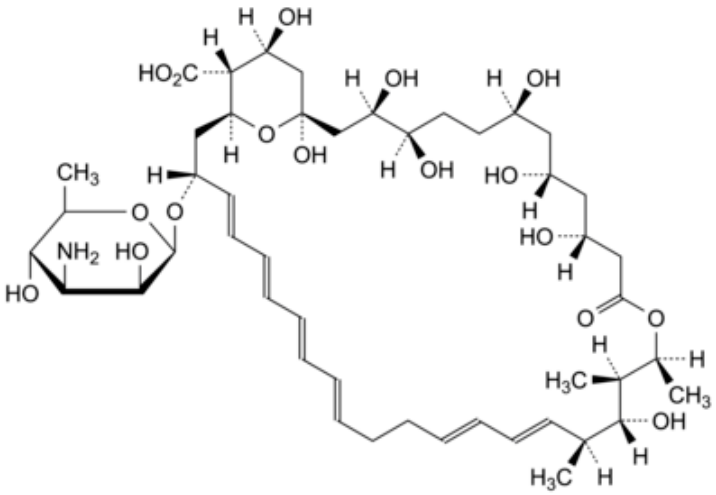
Edition: BP 2025 (Ph. Eur. 11.6 update)

Nystatin

General Notices

(Ph. Eur. monograph 0517)



$C_{47}H_{75}NO_{17}$ 926

Action and use

Antifungal.

Preparations

Nystatin Ointment

Nystatin Oral Suspension

Nystatin Vaginal Tablets

Nystatin Tablets

Ph Eur

DEFINITION

Antifungal substance obtained by fermentation using certain strains of *Streptomyces noursei* as the production micro-organism. It contains mainly tetraenes, the principal component being (1*S*,3*R*,4*R*,7*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*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]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid (nystatin A1).

Content

Minimum 4400 IU/mg (dried substance) and minimum 5000 IU/mg (dried substance) if intended for oral administration.

CHARACTERS

Appearance

Yellow or slightly brownish powder, hygroscopic.

Solubility

Practically insoluble in water, freely soluble in dimethylformamide and in dimethyl sulfoxide, slightly soluble in methanol, practically insoluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, E.

Second identification: A, C, D.

A. Examine the solution prepared in the test for absorbance between 220 nm and 350 nm (*2.2.25*). The solution shows 4 absorption maxima at 230 nm, 291 nm, 305 nm and 319 nm, and a shoulder at 280 nm. The ratios of the absorbances at the absorption maxima at 291 nm and 319 nm to the absorbance at the absorption maximum at 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. The ratio of the absorbance measured at the absorption maximum at 230 nm to that measured at the shoulder at 280 nm is 0.83 to 1.25.
B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *nystatin CRS*.

C. To about 2 mg add 0.1 mL of *hydrochloric acid R*. A brown colour develops.
D. To about 2 mg add 0.1 mL of *sulfuric acid R*. A brown colour develops that becomes violet on standing.
E. Examine the chromatograms obtained in the test for composition.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Absorbance (*2.2.25*)

Dissolve 0.10 g in a mixture of 5.0 mL of *glacial acetic acid R* and 50 mL of *methanol R* and dilute to 100.0 mL with *methanol R*. Dilute 1.0 mL of the solution to 100.0 mL with *methanol R*. Determined at the maximum at 305 nm within 30 min of preparation of the solution, the absorbance is not less than 0.60.

Composition

Liquid chromatography (2.2.29): use the normalisation procedure. *Carry out the test protected from light.*

Test solution Dissolve 20 mg of the substance to be examined in *dimethyl sulfoxide R* and dilute to 50 mL with the same solvent.

Reference solution (a) Dissolve 20 mg of *nystatin CRS* in *dimethyl sulfoxide R* and dilute to 50 mL with the same solvent.

Reference solution (b) Dissolve 20 mg of the substance to be examined in 25 mL of *methanol R* and dilute to 50 mL with *water R*. To 10.0 mL of the solution add 2.0 mL of *dilute hydrochloric acid R*. Allow to stand at room temperature for 1 h.

Reference solution (c) Dilute 1.0 mL of reference solution (a) to 100.0 mL with *dimethyl sulfoxide R*. Dilute 1.0 mL of this solution to 10.0 mL with *dimethyl sulfoxide R*.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm,

— *stationary phase: base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (5 µm),

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R*, 3.85 g/L solution of *ammonium acetate R* (29:71 *V/V*),

— *mobile phase B*: 3.85 g/L solution of *ammonium acetate R*, *acetonitrile R* (40:60 *V/V*),

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 35	100 → 0	0 → 100
35 - 45	0	100
45 - 50	0 → 100	100 → 0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 305 nm.

Injection 20 µL

Retention time Nystatin A1 = about 14 min.

System suitability Reference solution (b):

— *resolution*: minimum 3.5 between the 2 principal peaks (retention time = about 13 min and 19 min).

Composition:

— *nystatin A1*: minimum 85.0 per cent,

— *any other compound*: maximum 4.0 per cent,

— *disregard limit*: the area of the principal peak in the chromatogram obtained with reference solution (c); disregard any peak with a retention time of less than 2 min.

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 3.5 per cent, determined on 1.0 g.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). *Protect the solutions from light throughout the assay.*

Dissolve the substance to be examined and *nystatin CRS* separately in *dimethylformamide R* and dilute with a mixture of 5 volumes of *dimethylformamide R* and 95 volumes of buffer solution pH 6.0.

STORAGE

In an airtight container, protected from light.

LABELLING

The label states where applicable, that the substance is only for cutaneous use.

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