NATAMYCIN

Prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003) superseding specifications prepared at the 57th JECFA (2001) and published in FNP 52 Add 9 (2001) superseding specifications for pimaricin prepared at the 20th JECFA (1976), published in FNP 52 (1992). An ADI 0-0.3mg/kg bw was established at the 20th JECFA (1976).

SYNONYMS Pimaricin; INS No. 235

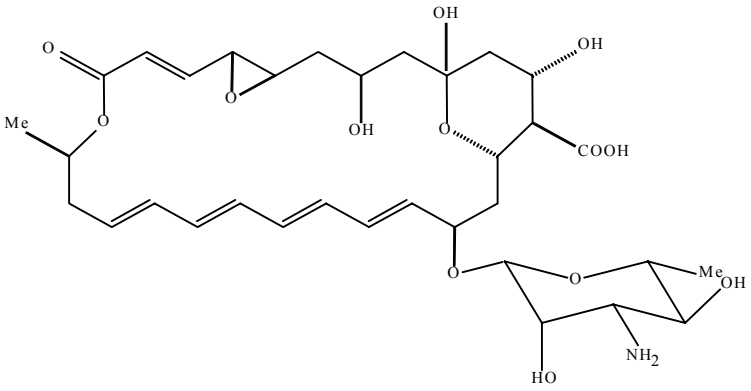
DEFINITION A fungicidal antimycotic of the polyene macrolide group. It is produced by several species of *Streptomyces*. The commercial product may contain up to three moles of water.

Chemical names A stereoisomer of 22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid

C.A.S. number 7681-93-8

Chemical formula $C_{33}H_{47}NO_{13}$

Structural formula



Formula weight 665.74

Assay Not less than 95.0% calculated on the dried basis

DESCRIPTION White to creamy-white, almost odourless, crystalline powder

FUNCTIONAL USES Fungicidal preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water, in lipid and in mineral oils; slightly soluble in methanol; soluble in glacial acetic acid and dimethylformamide.

Colour reaction On adding a few crystals of the sample, on a spot plate, to a drop of
- concentrated hydrochloric acid, a blue colour develops;

- concentrated phosphoric acid, a green colour develops, which changes into pale-red after a few minutes

Infrared absorption — The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in Appendix A.

Ultraviolet absorption — A solution of 5mg/l of the sample in 0.1% glacial acetic acid in methanol has absorption maxima at about 290, 303 and 318 nm, a shoulder at about 280 nm and exhibits minima at about 250, 295.5 and 311 nm. See Appendix B.

PURITY

Loss on drying (Vol. 4) — Not more than 8.0% (60°, over P_2O_5, pressure less than 5 mm Hg)

Specific rotation (Vol. 4) — $[\alpha]_D^{20}$: + 250° to + 295° (1% w/v solution in glacial acetic acid)

pH (Vol. 4) — 5.0 - 7.5 (1.0% w/v suspension in demineralised water)

Sulfated ash (Vol. 4) — Not more than 0.5%
Test 2 g of the sample (Method I)

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4,"Instrumental Methods"

METHOD OF ASSAY

High Performance Liquid Chromatography

(Note: Throughout this *Assay*, protect from direct light all solutions containing natamycin)

Mobile phase: Dissolve 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 ml of water, and mix. Add 5.0 ml of tetrahydrofuran and 240 ml of acetonitrile, mix, and filter through a 0.5-μm or finer porosity filter. Make adjustments if necessary to meet the system suitability requirements.

Standard preparation: Transfer about 20 mg of natamycin Reference Standard, accurately weighed, to a 100-ml volumetric flask. Add 5.0 ml of tetrahydrofuran, and sonicate for 10 min. Add 60 ml of methanol, and swirl to dissolve. Add 25 ml of water, and mix. Allow to cool to room temperature. Dilute with water to volume, mix, and filter through a membrane filter of 5-μm or finer porosity.

Resolution solution: To prepare a mixture of natamycin and natamycin methyl ester, dissolve 20 mg of natamycin in a mixture of 99 ml of methanol and 1 ml of 0.1 N hydrochloric acid, and allow to stand for 2 h.

Note: use this solution within 1 h.

Assay preparation: Transfer about 20 mg of natamycin, accurately weighed, to a 100-ml volumetric flask. Proceed as directed under "Standard preparation", beginning with "add 5.0 ml of tetrahydrofuran...."

Chromatographic system (see High-Performance Liquid Chromatography, (see Volume 4):

Use a high performance liquid chromatograph equipped with an ultraviolet detector measuring at 303 nm and a 4.6-mm x 25-cm column packed with octadecylsilanized silica (Supelcosil LC 18 or equivalent). The flow rate is about 3 ml/min. Chromatograph the "standard preparation", and record the peak responses. The column efficiency should not be less than 3000 theoretical plates and the tailing factor should be between 0.8 and 1.3. The relative standard deviation for three replicate injections of the standard preparation is not more than 1.0 %.

Chromatograph the "resolution solution". The relative retention times are about 0.7 for Natamycin and 1.0 for its methyl ester. The resolution (R) between Natamycin and its methyl ester is not less than 2.5:

$$R = 2(t_2-t_1)/(W_2+W_1)$$

where:
t_2 and t_1 are the retention times of natamycin methyl ester and natamycin, respectively

W_2 and W_1 are the width of the corresponding peaks at their bases extrapolated to the baseline.

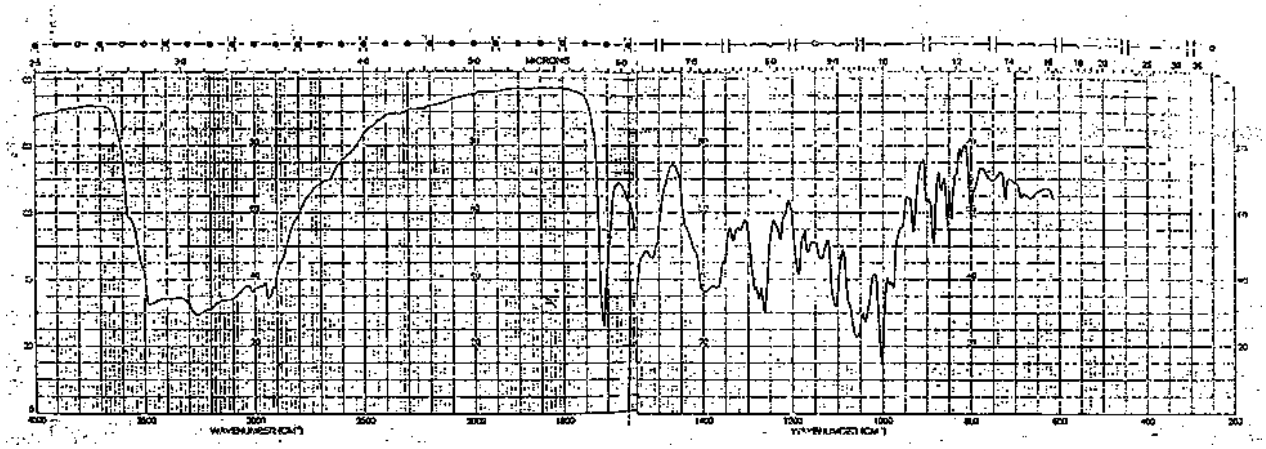
Procedure: Separately inject about 20 µl for each of the "standard preparation" and the "assay preparation" into the chromatograph, and record the peak areas of the major peaks. Calculate the percentage of Natamycin in the portion taken by the formula:

$$0.1(W_sP_s/W_u)(r_u/r_s)$$

in which W_s is the weight, in mg, of Natamycin Reference Standard taken to prepare the "Standard preparation); P_s is the stated content, in µg/ml, of Natamycin Reference Standard; W_u is the weight, in mg, of Natamycin taken to prepare the "Assay preparation"; and r_u and r_s are the peak area responses obtained with the "Assay preparation" and the "Standard preparation", respectively.

Appendix A

Reference Infrared Spectrum (1.3 mg solid in 300 mg potassium bromide) for natamycin



Appendix B

Ultraviolet absorption spectrum of natamycin
Concentration: 5 µg/ml in methanol/glacial acetic acid mixture