Food and Agriculture Organization of the United Nations

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Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 99th Meeting 2024

Natamycin

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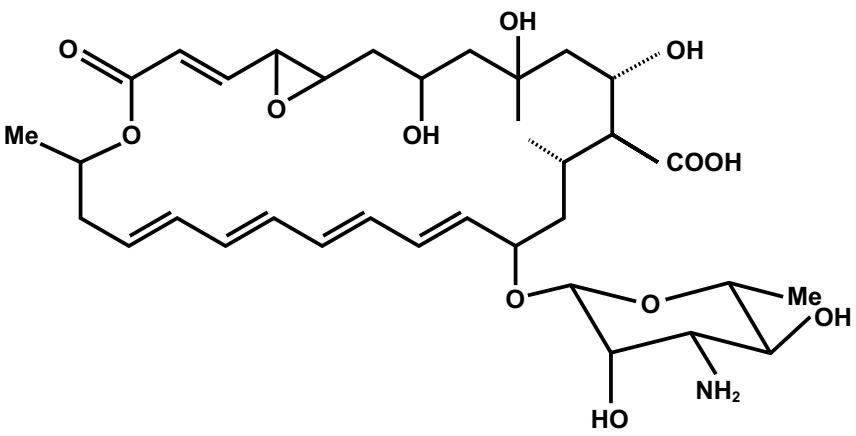
Natamycin

Revised specifications prepared at the 99th JECFA (2024) and published in FAO JECFA Monographs 34 (2025) superseding specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11 (2003). The Committee reaffirmed the ADI of 0- 0.3 mg/kg bw 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 (Main bands: 3 000-2 500 cm^{-1} Alcohol/Phenol O-H Stretch, 1716 cm^{-1} Carboxylic Acid C=O Stretch; 1570 cm^{-1} N-H bending of a primary amine).
Ultraviolet absorption (Vol. 4)	A solution of 5 mg/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.

PURITY

Loss on drying (Vol. 4)	Not more than 8.0% (60 °C, over P_2O_5, pressure less than 5 mm Hg)
Specific rotation (Vol. 4)	α_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 deionized water)
Sulfated ash (Vol. 4)	Not more than 0.5% Test 2 g of the sample (Method I)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine using a method 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
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method 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.

TESTS

METHOD OF ASSAY

Transglucosidase activity

The purity of natamycin can be determined both spectrophotometrically and by HPLC. Data must be expressed as percentage on dry basis. If the process can take to the formation of natamycin methyl ester, the HPLC method must be used and the resolution of the two peaks of natamycin and natamycin methyl ester must be guaranteed.

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

REAGENTS

Methanol (analytical or spectrophotometric grade, ≥99.9%); Aqueous methanol (mixing methanol and water (2+1 (v/v)); natamycin reference standard (minimum 95%).

NATAMYCIN STANDARD SOLUTIONS

Immediately before use, dissolve in a 100 ml volumetric flask, containing methanol, a known amount (50 mg) of standard natamycin, make up to the mark (solution A1); In a 50 ml volumetric flask, dilute 5 ml of solution A1 to 50 ml using the methanol-water solution (Solution B1); In a 50 ml volumetric flask, dilute 5 ml of solution B1 to 50 ml using the methanol-water solution to obtain the final standard natamycin (Solution C1): 5 mg/l of natamycin.

ASSAY PREPARATION

Immediately before use, dissolve in a 100 ml volumetric flask, containing methanol, a known amount (50 mg) of the unknown sample of natamycin (solution A2), proceed as described in Natamycin standard solution to obtain solutions B2 and C2 for the unknown sample.

Filter both solutions (standard and assay) through a syringe filter (0.45 μm) to obtain at least 3 ml of filtrate. Discharge the first drops.

SPECTROPHOTOMETRIC DETERMINATION

- **Calibration:** Record the spectrum of the Natamycin standard (Solution C1) in the range 300-340 nm, using the methanol-water solution as a blank. The spectrum should show a fingerprint with maxima at: 290, 303 and 318 nm, and minima at: 311 and 329 nm, respectively. Both the maximum and minimum values may vary slightly because of apparatus and solvents.
- **Sample solution:** Analogously to the standard solution, record the spectrum of the Natamycin unknown (Solution C2) in the range 300-340 nm, always using the methanol-water solution as a blank.
- Measure the absorbances, of both solutions, at 318 nm and at 311 and 329 nm, respectively.

- Read the net absorbances as:

$$\text{Net Abs} = \text{Abs318nm} - {}^{2}/_{3}\ \text{Abs311nm} - {}^{1}/_{3}\ \text{Abs329nm},$$

both for unknown sample and standard of natamycin.

- Calculate the concentration of natamycin in the unknown sample (mg/kg) as:

$$\text{Natamycin}\left(\frac{\text{mg}}{\text{kg}}\right) = \frac{\text{V(ml)}}{\text{Wsample(g)}} \times \frac{\text{NetAbs unknown sample}}{\text{NetAbs natamicin standard}} \times \text{natamicin standard}\left(\frac{\text{mg}}{\text{kg}}\right)$$

ALTERNATE METHOD

HPLC DETERMINATION:

Apparatus:

- HPLC equipped with an UV-Vis or Diode Array Detector (DAD); solvents HPLC purity grade.

Procedure:

- Mobile phase: 30% acetonitrile-70% acidified water (0.1% glacial acetic) v/v
- Flow rate: isocratic at 0.8 mi/min
- Detector: DAD, ranges 250-360 nm, Quantitative at 305 nm.
- Column: RP-18, 250 mmx4.6 mm id, 5µm packed material, complete of guard column.
- Expected Retention Time: about 6 minutes.
- **External HPLC calibration curve and retention time of natamycin:** Pipet, into a series of 50 ml volumetric flasks, 1, 2, 4, 6 and 8 ml of standard natamycin (solution C1) and make up to the mark with the methanol-water solution to obtain standard solutions containing: 0.1, 0.2, 0.4, 0.6 and 0.8 mg/l of natamycin, respectively. Filter through a syringe filter (0.45µm) and inject into the HPLC. Establish the retention time of natamycin and build a calibration curve. Express natamycin in mg/kg.
- **Sample injection:** Inject the same aliquot of the sample (solution C2) into the HPLC, measure the area of the corresponding natamycin peak. Substitute it in the calibration curve to obtain the corresponding concentration of natamycin in the unknown sample.
- Adjust the injection volumes and dilutions of the samples to ensure that the response remains within the linear response range of the DAD detector.

Appendix 1

Reference Infrared Spectrum for natamycin

(kindly provided by Database of Japan's Specifications and Standards for Food Additives)