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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 25532-2010

**National food safety standards of food additive natamycin
(English Translation)**

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Foreword

Appendix A of this standard is a normative appendix.

National Food Safety Standard Food additives Natamycin

1 Scope

This standard applies to the post-Natal Streptomyces (*Streptomyces natalensis*) controlled fermentation of food additives Natamycin.

2 Normative references

The standard file referenced in the application of this standard is essential. For cited documents with dates, only the date of Version applies to this standard. For undated references, the latest edition (including any amendments) applies to this standard.

3 Formula, relative molecular mass and structural formula

Formula 3.1

C₃₃H₄₇NO₁₃

3.3 Relative molecular mass

665.73 (according to 2007 international relative atomic mass)

4 Technical Requirements

4.1

Sensory requirements. comply with Table 1.

Table 1 Sensory requirements

Project requires test methods Color white to creamy yellow proper amount of sample is placed in a clean, dry white porcelain dish, natural Light, observe its color and texture, and smell its flavor. Odour almost odorless Organizational status crystalline powder

4.2

Physical indicators. to comply with Table 2.

Table 2. Physical and chemical indicators

Item Index Test Method Natamycin (dry basis), w /% ≥ 95.0 Appendix A A.3 Specific rotation α_D^{20} (20 °C, D)/[(°) · dm² · kg⁻¹] 250 ~ 295 Appendix A A.4 Loss on drying, w /% ≤ 8.0 GB 5009.3 vacuum drying method Residue on ignition, w /% ≤ 0.5 GB/T 9741a pH 5.0 ~ 7.5 in Appendix A A.5 Lead (Pb)/(mg/kg) ≤ 2 GB 5009.12 He said a sample of about 2g.

Appendix A

(Normative)

Testing method

A.1 General Provisions Unless otherwise indicated in the analysis using only recognized as analytical reagents and GB/T 6682-2008 specified in the water. In the analysis Standard titration solution, impurities measured by standard solution, preparations and products, did not indicate when the other requirements, according to GB/T 601, GB/T 602, the provisions of preparation GB/T 603's. This solution was used in the test does not indicate what is formulated with solvent, it refers to an aqueous solution.

A.2 Identification Test A.2.1 solubility Insoluble in water, slightly soluble in methanol. Dissolved in glacial acetic acid and dimethylformamide.

A.2.2 color reaction Take appropriate sample is placed in a dropping test board, dropping hydrochloric acid, to produce blue; dropping acid, to produce green, a few minutes later becomes reddish color. Dropping amount of acid to be able to completely wetted or dissolved natamycin is satisfied that the sample for the degree.

A.2.3 UV absorption spectrum Weigh accurately 25mg sample was transferred to 100mL volumetric flask, add 5.0mL water wet samples. Add 50mL glacial acetic acid - methanol solution Liquid [acetic acid. methanol = 11000 (. volume ratio)], in the absence of light and shaken until dissolved. And then glacial acetic acid - methanol solution to volume, Mix well. Pipette 2.0mL of the solution was placed in 100mL flask with glacial acetic acid - methanol solution to volume, and mix. The solution Ultraviolet absorption spectrum as compared with

the standard sample solution, having the same minimum and maximum absorption wavelength.

A.3 Determination of Natamycin A.3.1 Reagents and materials

- a) Natamycin standards.
- b) acetonitrile. chromatography.
- c) ammonium acetate.
- d) ammonium chloride.
- e) tetrahydrofuran.
- f) methanol.
- g) hydrochloric acid solution. 0.1mol/L.

A.3.2 Instruments and Equipment High performance liquid chromatograph. with UV detector (detection wavelength 303nm).

A.3.3 reference chromatographic conditions

- a) Column. 25cm x 4.6mm (diameter) column filled with octadecyl silica gel; or other equivalent column.
- b) Mobile phase. Weigh 3.0g of ammonium acetate and 1.0g of ammonium chloride, dissolved in 760mL of water, and mix. Add 5.0mL of tetrahydrofuran and 240mL Acetonitrile, mix and filter with 0.45µm membrane filter.
- c) Column temperature. room temperature.
- d) Mobile phase flow rate. 3mL/min.
- e) Injection volume. 20 µL

NOTE: System Suitability injection is repeated three times standard solution, the resulting response to the relative deviation of the mean area of less than 1.0%.

A.3.4 Analysis step

A.3.4.1 Preparation of standard solutions Weigh about 0.02g natamycin standard, accurate to 0.0001g, set 100mL flask, add 5.0mL of tetrahydrofuran, Ultrasonic vibration 10min. 60mL of methanol was added, rotate until dissolved. Was added 25mL of water, cooled to room temperature after mixing. Add water to the mark, mix and filter with 0.45µm membrane filter.

A.3.4.2 Preparation of sample solution Weigh about 0.02g sample (accurate to 0.0001g), was transferred to 100mL volumetric flask 5.0mL tetrahydrofuran, ultrasonic shock Swing 10min. 60mL of methanol was added, rotate until dissolved. Was added 25mL of water, cooled to room temperature after mixing. Add water to volume, mix Uniform, filtered through

a 0.45μm membrane filter.

A.3.4.3 Determination in A.3.3 reference chromatographic conditions, respectively, the standard solution and the sample solution were measured, recorded peak area, calculated according to the formula Sample natamycin content.

A.3.5 Calculation Results Natamycin content X_1 according to formula (A.1) Calculated.

X_1 0

CMX) (%) (A.1)

Where.

X_1 -- sample natamycin content,%;

MS-- natamycin standard mass in grams (g);

CS-- Natamycin standards in marked natamycin content,%;

MU-- sample mass, in grams (g);

X_0 -- sample measured loss on drying,%;

AU-- sample liquid chromatographic peak area obtained;

AS-- chromatographic analysis standard solution peak area obtained.

The results parallel arithmetic mean of the measurement results shall prevail. Twice in the same condition absolutely independent determination results the difference is not more than 2% of the arithmetic mean.

A.4 Determination of specific rotation A.4.1 weighed amount of sample in glacial acetic acid as solvent to prepare a sample solution of glacial acetic acid 10mg/mL, with the polarimeter is measured, While doing the blank control. The measurement temperature was $20\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$.

Specific rotation $\alpha_{\text{D}}(20\text{ }^{\circ}\text{C})$ value to $(^{\circ}) \cdot \text{dm}^2 \cdot \text{kg}^{-1}$ according to formula (A.2) Calculated.

Where.

α - measured angle of rotation, in degrees ($^{\circ}$);

l - the length of the optical tube unit decimeter (dm);

ρ

α

$D_{\text{Cm}} = ^{\circ}, 20$

ρ - mass concentration in the solution effective component in grams per milliliter