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

GB 29944-2013

**Food additive --
N-[N-(3,3-dimethyl-butyl)]-L-aspartyl-L-phenylalanine 1-methyl
ester (neotame)**

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(English Translation)

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Table of Contents

- 1 Scope
- 2 Chemical name, molecular formula, molecular mass and structural formula
 - 2.1 Chemical Name
 - 2.3 Formula
 - 2.4 Relative molecular mass
- 3 Technical requirements
 - 3.1 Sensory requirements
 - 3.2 Physical and Chemical Indicators

1 Scope

This standard applies to aspartame and 3,3-dimethyl butyraldehyde as the main raw material, in the presence of hydrogen in methanol solution was reacted after Methanol, washing and drying step of removing the food additive prepared N- [N- (3,3- dimethylbutyl)] - L-alpha- aspartyl -L- phenylalanine 1-A Ester (neotame).

2.1 Chemical Name

N- [N- (3,3- dimethylbutyl) -L-alpha- aspartyl] -L-phenylalanine 1-methyl ester
Formula 2.2
C 20 H30N2O5

2.4 Relative molecular mass

378.47 (according to 2007 international relative atomic mass)

3.1 Sensory requirements

Sensory requirements shall comply with the requirements of Table 1.

Table 1 Sensory requirements

Project requires test methods White to off-white color of the sample placed in a proper amount of white porcelain plate, observe its color under natural light Status and powder

3.2 Physical and Chemical Indicators

Physical and chemical indicators should be consistent with the provisions of Table 2.

Table 2. Physical and chemical indicators

Item Index Test Method Neotame content (dry basis), w /% 97.0 ~ 102.0 Appendix A A.3 N-[N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine, w /% <= 1.5 Appendix A A.4 Other related substances, w /% <= 2.0 A.5 in Appendix A Water, w /% <= 5.0 GB/T 6283 Residue on ignition, w /% <= 0.2 GB/T 9741 pH (5 g/L solution) 5.0 ~ 7.0 GB/T 9724 Lead (Pb)/(mg/kg) <= 1 GB 5009.12 Specific rotation α_D^{20} (20 °C, D)/[(°) · dm² · kg⁻¹] -40.0 ~ -43.3 GB/T 613 a formulated a mass fraction of 0.5% of the sample solution, the results on a dry basis.

Appendix A

Testing method

A.1 General Provisions Unless otherwise specified in this standard, the use of analytical reagent purity should be more than the standard titration solution, standard solution for measuring impurities, Preparations and products, should be GB/T 601, GB/T 602, the provisions of the preparation of GB/T 603, the test water should be consistent with GB/T 6682-2008 in three water Provisions. Solution was used in the tests did not indicate what is formulated with solvent, it refers to an aqueous solution.

A.2 Identification Test A.2.1 solubility Slightly soluble in water, soluble in ethanol.

A.2.2 IR

IR potassium bromide dispersion of the sample in line with the criteria in Appendix B in the infrared spectrum.

Determination A.3 Neotame Content A.3.1 Reagents and materials A.3.1.1 acetonitrile. chromatography.

A.3.1.2 sodium heptane. chromatography.

A.3.1.3 triethylamine. chromatography.

A.3.1.4 Water. GB/T 6682-2008 in a water.

A.3.1.5 phosphoric acid.

A.3.1.6 neotame standards.

A.3.2 Instruments and Equipment High performance liquid chromatograph. with UV detection, or other equivalent detectors.

A.3.3 reference chromatographic conditions A.3.3.1 Column. C18 column, 4.6 mm (inner diameter) x 100 mm (length), particle size 5 μm. Or other equivalent chromatographic separation Column and chromatographic conditions.

A.3.3.2 Mobile phase. A 3.0 g sodium dissolved in 740 mL of heptane water, adding 3.8 mL of triethylamine, with phosphoric acid to adjust the pH to 3.5, and then Diluted with water to 750 mL. Then add 250 mL of acetonitrile with phosphoric acid to adjust the pH to 3.7,

filtered spare.

A.3.3.3 Column temperature. 45 °C.

A.3.3.4 flow rate. 1.5 mL/min.

A.3.3.5 Injection volume. 20 µL.

A.3.3.6 detection wavelength. 210 nm.

A.3.4 Analysis step

A.3.4.1 Preparation of standard solutions Weighed amount neotame standard, accurate to 0.000 1 g, with flows to match to a concentration of about 1.0 mg/mL of the standard solution.

A.3.4.2 Preparation of sample solution Weighed amount of sample, accurate to 0.000 1 g, with flows to match to a concentration of about 1.0 mg/mL of the sample solution.

A.3.4.3 Determination in A.3.3 reference chromatographic conditions, respectively standard solution and sample solution chromatography. Chromatogram of the sample solution and standard solution records Neotame chromatogram peak area.

A.3.5 Calculation Results Neotame content mass fraction w_1 according to formula (A.1) Calculated.

$$1 \times x = c$$

r_w (A.1)

Where.

The main peak r_U -- sample solution chromatogram peak area;

The main peak r_S -- standard solution chromatogram peak area;

CS -- actual concentration of the standard solution, in milligrams per milliliter (mg/mL);

CU -- actual concentration of the sample solution, in milligrams per milliliter (mg/mL).

A.4 N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine determination A.4.1

Reagents and materials A.4.1.1 acetonitrile. chromatography.

A.4.1.2 sodium heptane. chromatography.

A.4.1.3 triethylamine. chromatography.

A.4.1.4 Water. GB/T 6682-2008 in a water.

A.4.1.5 N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine standard.

A.4.2 Instruments and Equipment with A.3.2.

A.4.3 reference chromatographic conditions with A.3.3.

A.4.4 Analysis step

A.4.4.1 Preparation of standard solutions Mobile phase (A.3.3.2) as solvent, weighed amount of N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine standard, exact to 0.000 1 g, formulated in a concentration of about 0.03 mg/mL of the standard solution.

A.4.4.2 Preparation of sample solution Mobile phase (A.3.3.2) as solvent, weighed amount of sample, accurate to 0.000 1 g, formulated in a concentration of about 2 mg/mL of the sample solution.

A.4.4.3 Determination in A.4.3 reference chromatographic conditions, respectively standard solution and sample solution chromatography. Chromatogram of the sample solution and standard solution records Chromatogram N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine peak area.

A.4.5 Calculation Results N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine mass fraction w_2 according to formula (A.2) Calculated.

$$2 \times x = c$$

r_w (A.2)

Where.

r_U -- sample solution chromatogram N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine peak area;

r_S -- standard solution chromatogram N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine peak area;

CS -- actual concentration of the standard solution, in milligrams per milliliter (mg/mL);

CU -- actual concentration of the sample solution, in milligrams per milliliter (mg/mL).

A.5 Determination of other related substances A.5.1 Reagents and materials A.5.1.1 acetonitrile. chromatography.

A.5.1.2 sodium heptane. chromatography.

A.5.1.3 triethylamine. chromatography.

A.5.1.4 Water. GB/T 6682-2008 in a water.

A.5.1.5 neotame standards.

A.5.1.6 N- [N- (3,3- dimethylbutyl) alpha] aspartyl] -L-phenylalanine standard.

A.5.2 Instruments and Equipment with A.3.2.

A.5.3 reference chromatographic conditions with A.3.3.

A.5.4 Analysis step