NEOTAME

New specifications prepared at the 61st JECFA (2003) and published in FNP 52 Add 11(2003). An ADI of 0 – 2 mg/kg bw was established at the 61st JECFA (2003).

SYNONYMS

INS No. 961

DEFINITION

Neotame is manufactured in single process in which aspartame and 3,3-dimethylbutyraldehyde are reacted together in a methanol solution in the presence of hydrogen. Neotame is isolated by removal of methanol, followed by washing and drying.

Chemical names

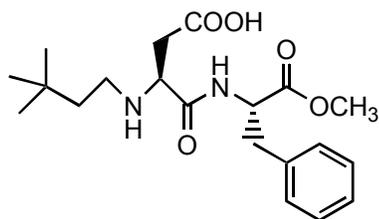N-[N-(3,3-Dimethylbutyl)-L-α-aspartyl]- L-phenylalanine 1-methyl ester

C.A.S. number

165450-17-9

Chemical formula

$C_{20}H_{30}N_2O_5$

Structural formula

Formula weight

378.47

Assay

Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION

White to off-white powder

FUNCTIONAL USES

Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Sparingly soluble in water, very soluble in ethanol

Infrared spectrum

The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.

PURITY

pH (Vol. 4)

5.0 – 7.0 (0.5 % solution)

Melting range (Vol. 4)

81° - 84°

Water (Vol. 4)

Not more than 5.0% in a sample size of 25$\pm$5 mg (Karl Fischer)

N-[N-(3,3-Dimethylbutyl)-α-aspartyl]-L-phenylalanine	Not more than 1.5% See under METHOD OF ASSAY
Other related substances	Not more than 2.0% based on the results of the Method of Assay using the following formula: $$100 \times A/(A+B)$$ where A = the sum of the peak areas for all secondary peaks other than those for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine and B = the sum of the peak areas for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine.
Sulfated ash (Vol. 4)	Not more than 0.2%
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between –40.0° and –43.3° (0.5 % solution) calculated on the anhydrous basis
Lead (Vol. 4)	Not more than 1 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 described in Volume 4, "Instrumental Methods".

METHOD OF ASSAY

Neotame

Determine by HPLC using the following conditions:
Mobile phase: 25% acetonitrile and 75% buffer (final pH of 3.7). The buffer is composed of 0.02 M heptanesulfonic acid sodium salt and 0.5% v/v triethylamine at pH 3.5.
Preparation of sample: Dissolve the sample in mobile phase solution to a concentration of 1 mg/ml.
Preparation of standard: Dissolve the neotame standard (NutraSweet Kelco) in mobile phase solution to a concentration of 1 mg/ml
HPLC Conditions:

Column:	Partisil 5 ODS3 (4.6 x 100 mm length) or equivalent.
Column temperature:	45°
Pump:	Isocratic
Solvent:	25% acetonitrile and 75% buffer adjusted to a pH of 3.7).
Flow rate:	1.5 ml/min
Injection:	25 µl
Detection:	UV 210 nm
Run Time:	approximately 18 min.

Calculation: Compare the area of the neotame peak in the sample (A_{sample}) to that in the standard ($A_{standard}$). Calculate the percentage content of the sample, on the dry basis, from the formula

$$\% \text{ neotame} = (A_{sample}/A_{standard}) \times 100 \times F$$

Where
F= 100/(100 - % water in sample)

N-[N-(3,3-Dimethylbutyl)-α-aspartyl]-L-phenylalanine

This is determined using the same HPLC method:
Preparation of sample: Dissolve the sample in mobile phase solution to a concentration of 2 mg/ml.
Preparation of standard: Dissolve the N-[N-(3,3-dimethylbutyl)-α-aspartyl]-L-phenylalanine standard (NutraSweet Kelco) in mobile phase solution to concentrations of 75, 45, 15, 3 and 0.9 μg/ml.

Calculation: The retention time for N-[N-(3,3-dimethylbutyl)-α-aspartyl]-L-phenylalanine is approximately 4.4 min compared with approximately 12.2 min for neotame.
Determine the area response of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine from the sample preparation. Prepare a full fit linear regression standard curve by plotting the area response of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the standard solution on the ordinate scale versus its respective concentration in μg/ml. From the slope and intercept of the standard curve, calculate the concentration, C_1 (μg/ml), of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the sample using the equation:

$$C_1 = (A_{sample} - \text{intercept})/\text{slope of curve}$$

Calculate the percentage of N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine in the sample using the equation

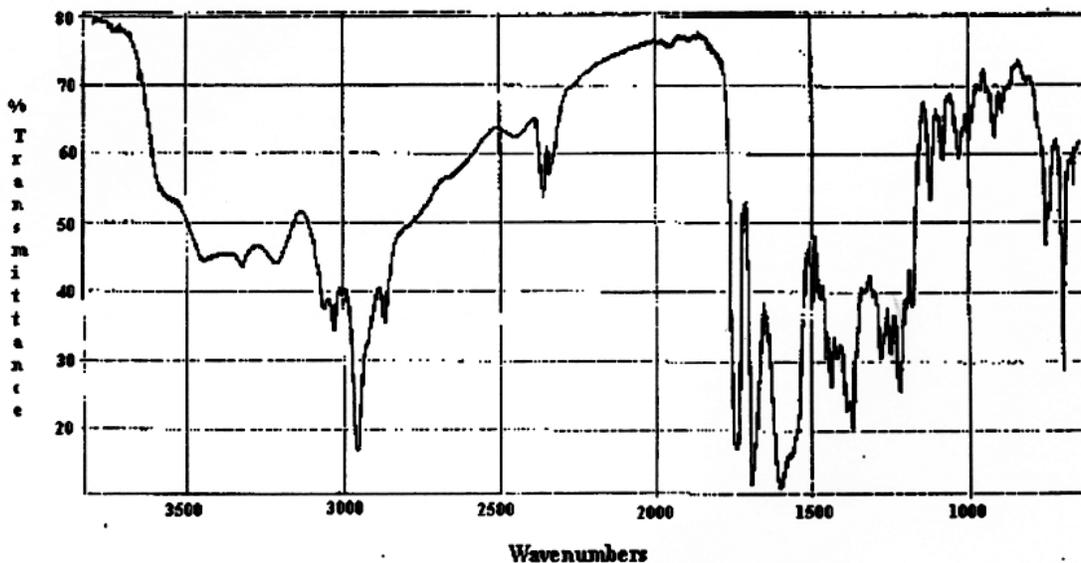% N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine = (C_1/C_2) x 100
where C_2 is the concentration of the sample.
See Appendix B for examples of chromatograms obtained using the method.

Appendix A   IR Spectrum of neotame standard

Wavenumbers

Chromatograms for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine (Std E1)