**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_{2}O_{5}

**STRUCTURAL FORMULA**

![Structural formula of Neotame]

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±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:

\[ 100 \times \frac{A}{(A+B)} \]

where:

\[ A = \text{the sum of the peak areas for all secondary peaks other than those for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine and} \]

\[ B = \text{the sum of the peak areas for neotame and N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine.} \]

Sulfated ash (Vol. 4)  Not more than 0.2%

Specific rotation (Vol. 4)  \([\alpha]_{20}^D\) : 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°C  
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 (\(A_{\text{sample}}\)) to that in the standard (\(A_{\text{standard}}\)). Calculate the percentage content of the sample, on the dry basis, from the formula

\[ \% \text{neotame} = \left( \frac{A_{\text{sample}}}{A_{\text{standard}}} \right) \times 100 \times F \]
Where
\[ F = \frac{100}{(100 - \% \text{ water in sample})} \]

**N-[N-(3,3-Dimethylbutyl)-\( \alpha \)-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)-\( \alpha \)-aspartyl]-L-phenylalanine standard (NutraSweet Kelco) in mobile phase solution to concentrations of 75, 45, 15, 3 and 0.9 \( \mu \)g/ml.

**Calculation:** The retention time for N-[N-(3,3-dimethylbutyl)-\( \alpha \)-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-\( \alpha \)-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-\( \alpha \)-aspartyl]-L-phenylalanine in the standard solution on the ordinate scale versus its respective concentration in \( \mu \)g/ml. From the slope and intercept of the standard curve, calculate the concentration, \( C_1 \) (\( \mu \)g/ml), of N-[N-(3,3-dimethylbutyl)-L-\( \alpha \)-aspartyl]-L-phenylalanine in the sample using the equation:

\[ C_1 = \frac{(A_{\text{sample}} - \text{intercept})}{\text{slope of curve}} \]

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

\[ \% \text{ N-[N-(3,3-dimethylbutyl)-L-\( \alpha \)-aspartyl]-L-phenylalanine} = \left( \frac{C_1}{C_2} \right) \times 100 \]

where \( C_2 \) is the concentration of the sample.

**Appendix A**

IR Spectrum of neotame standard
Appendix B

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