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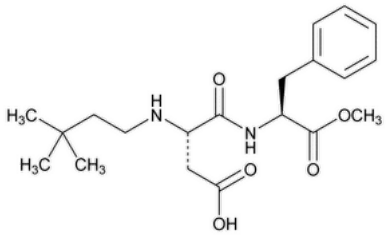
Neotame



$C_{20}H_{30}N_2O_5$ 378.46

L-Phenylalanine, *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-1-methyl ester;

N-[*N*-(3,3-Dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester CAS RN®: 165450-17-9.

DEFINITION

Neotame contains NLT 97.0% and NMT 102.0% of neotame ($C_{20}H_{30}N_2O_5$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

ASSAY

• **Procedure**

Mobile phase: Dissolve 3.0 g of sodium 1-heptanesulfonate in 740 mL of water in a suitable 1000-mL vessel, and add 3.8 mL of triethylamine. Adjust the resulting solution with phosphoric acid to a pH of 3.5, and dilute with water to 750 mL. Add 250 mL of acetonitrile, and adjust with phosphoric acid to an apparent pH of 3.7.

Standard solution: 1.0 mg/mL of USP Neotame RS in *Mobile phase*

Sample solution: 1.0 mg/mL of Neotame in *Mobile phase*. [Note—This solution is stable for up to 32 h when stored at a temperature of 0°–10°.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of neotame ($C_{20}H_{30}N_2O_5$) in the portion of Neotame taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Neotame RS in the *Standard solution* (mg/mL)

C_U = concentration of Neotame in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous basis

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.2%

• **Lead**

[Note—Use acid-cleaned (mixture of 5% nitric acid and 5% hydrochloric acid followed by rinsing with water) autosampler cups and volumetric glassware to avoid contamination. For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable. Store standards and samples in acid-cleaned polyethylene containers.]

Diluent: Transfer 2 mL of lead-free nitric acid into a 1000-mL volumetric flask, dilute with water to volume, and mix.

Standard stock solution: 79.9 mg of lead nitrate in 100 mL of *Diluent* in a 500-mL volumetric flask, and dilute with *Diluent* to volume. Transfer 10.0 mL of the resulting solution into a 100-mL volumetric flask, and dilute with *Diluent* to volume. Each mL of the *Standard stock solution* contains the equivalent of 10 µg of lead.

Standard solution A: Dilute an aliquot of the *Standard stock solution* with *Diluent* to obtain a solution having a concentration of 0.03 µg/mL.

Standard solution B: Dilute an aliquot of the *Standard stock solution* with *Diluent* to obtain a solution having a concentration of 0.015 µg/mL.

Sample solution: Transfer 160 mg of Neotame to a 10-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume.

Blank: *Diluent*

Instrumental conditions

(See *Atomic Absorption Spectroscopy ⟨852⟩*.)

[Note—Optimize the instrument program as recommended by the manufacturer for lead, using a char temperature of 500° and an atomization temperature of 2000°.]

Mode: Atomic absorption spectrophotometer with a graphite furnace, pyrolytically coated graphite tubes, a solid pyrolytic graphite platform, and a background compensation system

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Purge gas: Argon

Alternate gas: Breathing-quality air

Volume: 15 µL

Analysis

Samples: *Standard solution A*, *Standard solution B*, *Sample solution*, and *Blank*

Correct the peak areas of the *Sample solution*, *Standard solution A*, and *Standard solution B* for the *Blank* peak area. Generate the appropriate lead calibration algorithm, and determine the lead concentration in the *Sample solution*, in µg/mL.

Calculate the amount of lead, in µg/g, in the portion of Neotame taken:

$$\text{Result} = (C \times V)/(W \times F)$$

C = blank-corrected lead concentration in the *Sample solution* (µg/mL)

V = volume of the *Sample solution*, 10 mL

W = weight of Neotame taken to prepare the *Sample solution* (mg)

F = conversion factor, mg/g

Acceptance criteria: NMT 2 µg/g

• **Related Compounds**

Mobile phase and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution A: 0.03 mg/mL of USP Neotame Related Compound A RS in *Mobile phase*

Standard solution B: Prepare as directed for the *Standard solution* in the *Assay*.

Detector sensitivity solution: Transfer 2 mL of *Standard solution A* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: 2 mg/mL of Neotame in *Mobile phase*. [Note—This solution is stable for up to 32 h when stored at a temperature of 0°–10°.]

System suitability

Samples: *Standard solution A* and *Detector sensitivity solution*

Suitability requirements

Relative standard deviation: NMT 5.0%, *Standard solution A*

Signal-to-noise ratio: NLT 10, *Detector sensitivity solution*

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of neotame related compound A in the portion of Neotame taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of neotame related compound A from the *Sample solution*

r_S = peak response of neotame related compound A from *Standard solution A*

C_S = concentration of USP Neotame Related Compound A RS in *Standard solution A* (mg/mL)

C_U = concentration of Neotame in the *Sample solution* (mg/mL)

Calculate the percentage of other impurities in the portion of Neotame taken:

$$\text{Result} = (r_T/r_S) \times (C_S/C_U) \times 100$$

r_T = sum of the peak responses of all impurities (except those of neotame related compound A and the solvent, if observed) from the *Sample solution*

r_S = peak response of neotame from *Standard solution B*

C_S = concentration of USP Neotame RS in *Standard solution B* (mg/mL)

C_U = concentration of Neotame in the *Sample solution* (mg/mL)

Acceptance criteria

Neotame related compound A: NMT 1.5%

Other impurities: NMT 2.0%

SPECIFIC TESTS

- **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*

  Sample solution: 5 mg/mL in water

  Acceptance criteria: −40.0° to −43.4°, at 20°

- **Water Determination** ⟨921⟩, *Method I, Method Ia*

  Sample: 0.50 g

  Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, store in a dry place, and avoid exposure to excessive heat.

- **USP Reference Standards** ⟨11⟩

  USP Neotame RS

  USP Neotame Related Compound A RS

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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
NEOTAME	Documentary Standards Support	SE2020 Simple Excipients

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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