GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard -

Determination of neotame in foods

食品安全国家标准

食品中纽甜的测定

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National food safety standard - Determination of neotame in foods

1 Scope

This Standard specifies the high performance liquid chromatography for the determination of neotame content in foods.

This Standard applies to the determination of neotame in beverages, preserved fruits, pastries, roasted seeds and nuts, pickled vegetables, candies, jams, jellies and compound seasonings.

2 Principle

After sample is extracted using mixed extracting solution and purified using solid phase extraction column, use the high performance liquid chromatograph for determination, using the retention time for quantitation and the peak area external standard for quantitation.

3 Reagents and materials

Unless specified otherwise, all reagents used are analytically pure and the water is grade one water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Acetonitrile (CH_3CN): chromatographically pure.

3.1.2 1-Octanesulfonic acid sodium salt ($C_8H_{17}NaO_3S$): chromatographically pure.

3.1.3 Phosphoric acid (H_3PO_4).

3.1.4 Formic acid (CH_3COOH): chromatographically pure.

3.1.5 Methanol (CH_3OH): chromatographically pure.

3.1.6 Triethylamine ($C_6H_{15}N$): chromatographically pure.

3.2 Preparation of reagents

3.2.1 Mixed extract: absorb 0.8 mL of formic acid and 2.5 mL of triethylamine; add water to make up to 1,000 mL. The pH is about 4.5.

3.2.2 Ion pair reagent buffer solution: weigh 2.00 g of 1-Octanesulfonic acid sodium salt; use 500 mL of water to dissolve; add 1.0 mL of phosphoric acid; add water to make up to 1,000 mL.

3.3 Standard substance

Neotame ($C_{20}H_{30}N_2O_5$, CAS no.: 165450-17-9), of purity ≥ 99.0%.

3.4 Preparation of standard solutions

3.4.1 Standard stock solution: weigh accurately 0.100 0 g of neotame standard substance; add mixed extracting solution to dissolve and make up to 100 mL. The neotame content of this solution is 1.00 mg/mL.

3.4.2 Standard working solutions: absorb an appropriate amount of neotame standard stock solution; use mixed extracting solution to prepare serial standard working solutions of 0.2 μg/mL, 1.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL, 50.0 μg/mL and 100.0 μg/mL.

3.5 C_{18} solid phase extraction column: 6 mL, 500 mg or equivalent and activated before use, using 5 mL of methanol and 10 mL of water in succession.

3.6 Filter membrane 0.45 μm, organic system.

4 Apparatus

4.1 Liquid chromatograph, which is equipped with an ultraviolet detector or a diode array detector.

4.2 Ultrasonic cleaner.

4.3 Analytical balance of sensitivities 0.000 1 g and 0.01 g.

4.4 Blender.

4.5 Vortex oscillator.

4.6 Nitrogen blowing concentrator.

4.7 Solid phase extraction apparatus.

4.8 Centrifugal machine: of rotational speed ≥ 4,000 r/min.

5 Analytical procedures

5.1 Sample preparation

5.1.1 Solid sample

Weigh 10 g (accurate to 0.01 g) of sample pulverized evenly to place into a stoppered plastic centrifugal tube of 50 mL; add 30 mL of mixed extract; carry out vortex oscillation for 10 min and ultrasonic extraction for 30 min; use mixed extracting solution to make up to scale. If the solution is turbid, filter it after centrifugation for 10 min at not lower than 4,000 r/min.

5.1.2 Liquid sample

Weigh accurately 10.0 mL of sample to place into a stoppered plastic centrifugal tube of 50 mL; add 30 mL of mixed extracting solution; carry out vortex oscillation to mix up. After ultrasonic extraction for 15 min, use mixed extracting solution to make up to scale. If the solution is turbid, filter it after centrifugation for 10 min at not lower than 4,000 r/min.

NOTE: First slightly heat gas-containing sample such as carbonated beverages and aerated waters; stir to remove carbon dioxide in sample or carry out ultrasonic degassing; then accurately measure and take sample.

5.2 Sample purification

Absorb 10.0 mL of filtrate to pass the solid phase extraction column at the flow rate of 1 mL/min ~ 2 mL/min; after the filtrate is fully discharged, use 5 mL of mixed extracting solution to elute the extraction column at the flow rate of 1 mL/min ~ 2 mL/min; discard all effluent; use 5 mL of methanol to elute at the flow rate of 1 mL/min; use the nitrogen blowing concentrator for the concentration of eluant on a water bath at 40°C; use mixed extracting solution to make up to 2.0 mL; filter it using filter membrane of 0.45 μm before using it as the solution to be tested on the liquid chromatograph.

5.3 Instrument reference conditions

5.3.1 Chromatographic column: C_{18} chromatographic column, 5 μm, 250 mm × 4.6 mm (inner diameter) or equivalent.

5.3.2 Column temperature: 30°C.

5.3.3 Moving phase: phase A: acetonitrile; phase B: ion pair reagent buffer solution. For the gradient elution procedures see Table 1.

5.3.4 Flow rate: 1.0 mL/min.

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