GB

NATIONAL STANDARD OF THE

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National food safety standard - Determination of nonylphenol in food

食品安全国家标准 食品中壬基酚的测定

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1 Scope

This Standard specifies the liquid chromatography-tandem mass spectrometry method for the determination of 4-nonylphenol in food.

This Standard applies to the determination of 4-nonylphenol in food.

2 Principle

4-nonylphenol in the sample is extracted with acetonitrile or acetonitrile solution, separated by high performance liquid chromatography, detected by tandem mass spectrometer, and quantified by isotope internal standard method.

3 Reagents and materials

Unless otherwise specified, the reagents used in this method are of chromatographic purity, and the water is grade 1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_3OH).

3.1.2 Acetonitrile (CH_3CN).

3.1.3 Ammonia water ($NH_3 \cdot H_2O$): concentration ≥ 25 %.

3.2 Reagent preparation

3.2.1 70 % acetonitrile solution: measure 700 mL of acetonitrile, dilute to 1000 mL with water.

3.2.2 0.1 % ammonia solution: pipette 1 mL of ammonia solution, dilute to 1000 mL with water.

3.3 Standards

3.3.1 4-nonylphenol ($C_{15}H_{24}O$) standard

4-nonylphenol ($C_{15}H_{24}O$, CAS No.: 84852-15-3), purity ≥ 99 %, or a standard certified by the state and awarded a reference standard certificate.

3.3.2 Isotope internal standard (3,6,3-nonylphenol-$^{13}C_6$)

3,6,3-nonylphenol-$^{13}C_6$ standard ($^{13}C_6C_9H_{24}O$, CAS No.: 1173020-38-6), purity ≥ 99 %.

3.4 Preparation of standard solutions

3.4.1 4-nonylphenol standard stock solution (100 mg/L): accurately weigh 10 mg of 4-nonylphenol standard (accurate to 0.00001 g), place in a 10 mL beaker, dissolve in methanol, transfer to a 100 mL brown volumetric flask, make up to the mark with methanol, mix well, store at -18 ℃, the shelf life is 6 months.

3.4.2 Isotope internal standard stock solution (100 mg/L): accurately weigh 10 mg of 3,6,3-nonylphenol-$^{13}C_6$ standard (accurate to 0.00001 g), place in a 10 mL beaker, dissolve in methanol, transfer to a 100 mL brown volumetric flask, make up to the mark with methanol, mix well, store at -18 ℃, the shelf life is 6 months.

3.4.3 4-nonylphenol standard intermediate solution (1.0 mg/L): accurately transfer 1.0 mL of 4-nonylphenol standard stock solution (100 mg/L) into a 100 mL brown volumetric flask, make up to the mark with methanol, mix well, store at -18°C, the shelf life is 3 months.

3.4.4 Isotope internal standard working solution (1.0 mg/L): accurately transfer 1.0 mL of isotope internal standard stock solution (100 mg/L) into a 100 mL brown volumetric flask, make up to the mark with methanol, mix well, store at -18°C, the shelf life is 3 months.

3.4.5 4-nonylphenol standard series working solutions: accurately pipette 2.50 mL of 4-nonylphenol standard intermediate solution (1.0 mg/L) into 25 mL brown volumetric flasks, dilute with methanol and make up to the mark, shake well, then respectively pipette 100 μL, 250 μL, 500 μL, 1.25 mL, 2.50 mL, 5.00 mL of the above solutions and 125 μL of isotope internal standard intermediate solution (1.0 mg/L), place into 25 mL brown volumetric flasks, make up to the mark with acetonitrile, and mix well. The mass concentrations of 4-nonylphenol are 0.40 ng/mL, 1.00 ng/mL, 2.00 ng/mL, 5.00 ng/mL, 10.0 ng/mL, and 20.0 ng/mL respectively, and the mass concentration of isotope internal standard solution in the standard working solution is 5.0 ng/mL. Prepare before use.

3.5 Materials

3.5.1 Polytetrafluoroethylene centrifuge tubes: 5 mL and 10 mL.

4 Instruments and equipment

4.1 Liquid chromatography-tandem mass spectrometer: equipped with electrospray ion source.

4.2 Analytical balance: the sensitivity is 0.00001 g and 0.01 g respectively.

and 5.0 mL of 70 % acetonitrile solution (5.0 mL of acetonitrile for meat and aquatic samples) in sequence, vortex for 30 s, seal with a stopper and ultrasonically extract for 30 min, cool to room temperature, centrifuge at 10000 r/min for 5 min at 4 ℃, pipette all the upper layer solution into a 5 mL volumetric flask, make up to the mark with acetonitrile, mix well; if the test solution is still turbid, centrifuge once more, take the supernatant and determine by liquid chromatography-tandem mass spectrometry.

6.1.4 Milk powder and infant formula

Weigh 1 g of sample (accurate to 0.01 g) into a 5 mL glass graduated tube, add 25.0 μL of isotope internal standard working solution (1.0 mg/L) and about 1.5 mL of hot water at about 50 ℃ in sequence, vortex for 10 s to fully dissolve, ultrasonically extract for 10 min, add 3.5 mL of acetonitrile, seal with a stopper and shake vigorously for 5 s, pipette all the upper layer solution and transfer to a 5 mL polytetrafluoroethylene centrifuge tube, centrifuge at 10000 r/min for 5 min at 4 ℃, pipette all the upper layer solution and place in a 5 mL volumetric flask, make up to the mark with acetonitrile, mix well; if the test solution is still turbid, centrifuge once more, take the supernatant and determine by liquid chromatography-tandem mass spectrometry.

6.2 Instrument reference conditions

6.2.1 Liquid chromatography reference conditions:

a) Chromatographic column: C_{18} chromatographic column (4.6 mm × 50 mm, 1.8 μm), equipped with pre-column (4.6 mm × 5 mm, 1.8 μm), or columns with equivalent performance;

b) Mobile phase: 0.1 % ammonia solution + methanol = 10 + 90 (volume ratio);

c) Flow rate: 0.35 mL/min;

d) Column temperature: 30 ℃;

e) Sample injection volume: 5 μL.

6.2.2 Mass spectrometry reference conditions:

a) Ionization mode: electrospray ionization negative ion mode (ESI^-);

b) Mass spectrometry scanning mode: multiple reaction monitoring (MRM);

c) Drying gas temperature: 340 ℃ (nitrogen);

d) Drying gas flow rate: 8 L/min (nitrogen);

e) Nebulizer pressure: 40 psi;

f) Capillary voltage: 4000 V;

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