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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.302-2025

**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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State Administration for Market Regulation.**

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National food safety standard - Determination of nonylphenol in food

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 ($\text{NH}_3 \cdot \text{H}_2\text{O}$): concentration $\geq 25\%$.

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 ($\text{C}_{15}\text{H}_{24}\text{O}$) standard

4-nonylphenol ($\text{C}_{15}\text{H}_{24}\text{O}$, CAS No.: 84852-15-3), purity $\geq 99\%$, or a standard certified by the state and awarded a reference standard certificate.

3.3.2 Isotope internal standard (3,6,3-nonylphenol- $^{13}\text{C}_6$)

3,6,3-nonylphenol- $^{13}\text{C}_6$ standard ($^{13}\text{C}_6\text{C}_9\text{H}_{24}\text{O}$, CAS No.: 1173020-38-6), purity $\geq 99\%$.

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 $-18\text{ }^{\circ}\text{C}$, 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- $^{13}\text{C}_6$ 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 $-18\text{ }^{\circ}\text{C}$, 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 $-18\text{ }^{\circ}\text{C}$, 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 $-18\text{ }^{\circ}\text{C}$, 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 °C, 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 μ L of isotope internal standard working solution (1.0 mg/L) and about 1.5 mL of hot water at about 50 °C 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 °C, 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: C₁₈ chromatographic column (4.6 mm $\times$ 50 mm, 1.8 μ m), equipped with pre-column (4.6 mm $\times$ 5 mm, 1.8 μ m), 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 °C;
- e) Sample injection volume: 5 μ L.

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 °C (nitrogen);
- d) Drying gas flow rate: 8 L/min (nitrogen);
- e) Nebulizer pressure: 40 psi;
- f) Capillary voltage: 4000 V;

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