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National food safety standard - Determination of N-nitrosamines in foods

食品安全国家标准 食品中 N-亚硝胺类化合物的测定

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Table of Contents

National food safety standard -

Determination of N-nitrosamines in foods

1 Scope

This standard specifies the method for the determination of N-dimethylnitrosamine (NDMA) in foods.

This standard applies to the determination of N-dimethylnitrosamine in meat and meat products, aquatic animals and their products.

Method 1: Steam distillation-gas chromatography-mass spectrometry/mass spectrometry

2 Principle

This method uses N-dimethylnitrosamine-D_6 as the internal standard. The internal standard is added to the sample; after steam distillation, the N-dimethylnitrosamine in the sample is absorbed by dichloromethane and separated by liquid-liquid extraction, which is determined by gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) and quantified by the internal standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-1 water specified by GB/T 6682.

3.1 Reagents

3.1.1 Dichloromethane (CH_2Cl_2): chromatographic pure.

3.1.2 Concentrated sulfuric acid (H_2SO_4): 18.4 mol/L.

3.1.3 Isooctane (C_8H_{18}): chromatographic pure.

3.1.4 Anhydrous sodium sulfate (Na_2SO_4).

3.1.5 Sodium chloride (NaCl): guaranteed reagent.

3.2 Preparation of reagents

Sulfuric acid solution (1+3): Measure 30 mL of concentrated sulfuric acid; slowly pour it into 90 mL of cold water; stir to fully dissipate heat; mix carefully after cooling.

3.3 Standards

3.3.1 N-dimethylnitrosamine standard solution ($C_2H_6N_2O$, CAS number: 62-75-9): N-dimethylnitrosamine methanol solution with a mass concentration of 1 000 μg/mL, or standard products certified by the nation and awarded a standard material certificate.

3.3.2 N-dimethylnitrosamine-D_6 internal standard solution (NDMA-D_6, $C_2D_6N_2O$, CAS number: 17829-05-9): the mass concentration is 1 000 μg/mL, and the solvent is methanol.

3.4 Preparation of standard solutions

3.4.1 N-dimethylnitrosamine standard stock solution (100 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine standard solution (1 000 μg/mL); put it in a 10 mL volumetric flask; use dichloromethane to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container; store at -18 °C in the dark for 6 months.

3.4.2 N-dimethylnitrosamine standard intermediate solution (1 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine standard stock solution (100 μg/mL); place it in a 100 mL volumetric flask; use dichloromethane to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container; store at -18 °C in the dark for 3 months.

3.4.3 N-dimethylnitrosamine-D_6 internal standard stock solution (100 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine-D_6 standard solution; place it in a 10 mL volumetric flask; use dichloromethane to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container; store at -18 °C in the dark for 6 months.

3.4.4 N-dimethylnitrosamine-D_6 internal standard intermediate solution (1 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine-D_6 internal standard stock solution; place it in a 100 mL volumetric flask; use dichloromethane to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container; store at -18 °C in the dark for 3 months.

3.4.5 N-dimethylnitrosamine standard and internal standard mixed series working solutions: Respectively and accurately draw 0.1 mL, 0.2 mL, 0.5 mL, 1.0 mL and 2.0 mL of N-dimethylnitrosamine standard intermediate solution (1 μg/mL); place them in the 10 mL volumetric flasks; add 0.4 mL of internal standard intermediate solution (1 μg/mL) to each; use dichloromethane to adjust the volume to the mark; mix well. The mass concentrations of N-dimethylnitrosamine standard series working solutions are 10 μg/L, 20 μg/L, 50 μg/L, 100 μg/L and 200 μg/L, among which the mass concentrations of the internal standards are all 40 μg/L. Prepare when necessary.

4 Instruments and apparatuses

4.1 Gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS).

4.2 Rotary evaporator.

4.3 All-glass steam distillation device or fully automatic steam distillation device (see Figure A.1 in Appendix A).

4.4 Nitrogen-blowing instrument.

4.5 Electric balance: sensitivity of 0.001 g.

4.6 Ice maker.

4.7 Cooling water preparation machine.

4.8 Water bath.

4.9 10 mL graduated test tube.

4.10 100 μL ~ 1 000 μL pipette or graduated pipette.

5 Analysis steps

5.1 Sample pretreatment

5.1.1 Sample preparation

Take the edible part of the representative sample and mash it; prepare it into a uniform sample; put it into a clean container; seal it and mark it well. Freeze and store the sample at -18 °C for future use.

5.1.2 Extraction

Accurately weigh 20 g (accurate to 0.01 g) of the sample; add 40 μL of N-dimethylnitrosamine internal standard intermediate solution (1 μg/mL); add 100 mL of water and 50 g of sodium chloride into the distillation bottle (tube); mix thoroughly. Air tightness inspection is required before and after loading the sample. Add 50 mL of dichloromethane and 0.5 mL of isooctane into a 250 mL Erlenmeyer flask; extend the outlet of the condenser tube (cooling water temperature controlled at 10 °C ~ 15 °C) below the dichloromethane liquid surface; place the Erlenmeyer flask in an ice bath; turn on the distillation device for heating and distillation (reference conditions: steam power of the automatic distillation device set to 50%); collect 200 mL ~ 250 mL of condensate (including 50 mL of dichloromethane extract); then, turn off the heating device and stop distillation.

corresponding N-dimethylnitrosamine mass concentration calculated from the standard curve, in micrograms per liter (μg/L);

V – the final constant volume of the test solution, in milliliters (mL);

1 000 – conversion coefficient;

m – sample mass, in grams (g).

Retain 2 digits after the decimal point for the calculation results.

7 Precision

The absolute difference of 2 independent test results obtained under repeatability cannot exceed 20% of the arithmetic mean value.

8 Others

8.1 Method detection limit

When the weighing amount is 20 g, and the constant volume is 1.0 mL, the detection limit of this method is 0.30 μg/kg, and the quantitation limit is 1.00 μg/kg.

8.2 Safety warning

The reagents involved in this test that may cause harm to the human body include organic solvents (such as acetonitrile, n-hexane), etc. Test personnel may be at risk due to exposure to these substances. Therefore, test operations involving these substances shall be conducted in a fume hood and professional protective tools (such as gloves, masks, etc.) shall be worn.

Nitrosamine compounds are strong carcinogens. After the test is completed, discarding these substances at will may cause damage to personnel and the environment. At the same time, if handled improperly, the N-dimethylnitrosamine standard used in the test and the expired standard substances may also bring risks. Therefore, these substances must not be discarded at will during the test; they shall be recovered in closed containers and stored in a well-ventilated environment away from fire sources.

Method 2: QuEChERS-gas chromatography-mass spectrometry/mass spectrometry

9 Principle

This method uses N-dimethylnitrosamine-D_6 as the internal standard. The internal standard is added to the sample; the N-dimethylnitrosamine in the sample is extracted

with acetonitrile. The matrix dispersion extraction filler is used for purification. Gas chromatograph-mass spectrometer/mass spectrometer is used for determination, and internal standard method for quantification.

10 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water specified by GB/T 6682.

10.1 Reagents

10.1.1 Acetonitrile (C_2H_3N): chromatographic pure.

10.1.2 Magnesium sulfate ($MgSO_4$).

10.1.3 Sodium chloride (NaCl): guaranteed reagent.

10.1.4 PLS-A powder: pyrrolidone modified polystyrene divinylbenzene polymer powder or EMR-Lipid extraction powder: enhanced degreasing dispersion powder.

10.1.5 Microporous membrane (0.22 μm), organic phase type.

10.2 Standards

Same as 3.3.

10.3 Preparation of standard solutions

10.3.1 N-dimethylnitrosamine standard stock solution (100 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine standard solution; place it in a 10 mL volumetric flask; use acetonitrile to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container. Store at -18 °C away from light, with a shelf life of 6 months.

10.3.2 N-dimethylnitrosamine standard intermediate solution (1 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine standard stock solution (100 μg/mL); place it in a 100 mL volumetric flask; use acetonitrile to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container. Store at -18 °C away from light, with a shelf life of 3 months.

10.3.3 N-dimethylnitrosamine-D_6 internal standard stock solution (100 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine-D_6 standard solution; place it in a 10 mL volumetric flask; use acetonitrile to adjust the volume to the mark; mix well. Transfer the solution to a brown glass container. Store at -18 °C away from light, with a shelf life of 6 months.

10.3.4 N-dimethylnitrosamine-D_6 internal standard intermediate solution (1 μg/mL): Accurately draw 1.0 mL of N-dimethylnitrosamine-D_6 internal standard stock solution;

centrifuge; rotate at 9 000 r/min, and centrifuge at 10 °C for 5 minutes. The supernatant needs to be purified.

12.1.3 Purification

Weigh 150 mg of PLS-A powder (or 1 g of enhanced lipid removal EMR-Lipid extraction powder or equivalent) into a 15 mL centrifuge tube; add 5 mL of water; vortex; immediately add 5 mL of the supernatant in 12.1.2 and vortex 1 min; place it in refrigerated centrifuge; centrifuge at 9 000 r/min, at 10 °C for 5 min.

12.1.4 Water removal

Weigh 1.6 g of magnesium sulfate and 0.4 g of sodium chloride into another 15 mL centrifuge tube; add the purification solution in 12.1.3; vortex for 2 minutes; place in a refrigerated centrifuge; rotate at 9 000 r/min, and centrifuge at 10 °C for 5 min. Take the upper organic phase and filter it through a 0.22 μm microporous membrane; then, put it on the machine for measurement.

Note: The test must be conducted in a fume hood and professional protective tools (such as masks, gloves, etc.) must be worn.

12.2 Apparatus reference conditions

12.2.1 Gas chromatographic conditions

a) Chromatographic column: highly polar quartz capillary column [30 m × 0.25 mm (inner diameter) × 0.25 μm (film thickness)], or equivalent.

b) Inlet temperature: initial temperature 50 °C, kept for 0.16 min, increased to 220°C at 900 °C/min and kept for 5 min.

c) Inlet solvent vent mode: vent time 0.16 min, vent flow 50 mL/min.

d) Carrier gas: helium, purity ≥99.999%, flow rate 1 mL/min.

e) Injection method: splitless injection.

f) Injection volume: 5 μL.

g) Temperature programming: initial temperature 40 °C, increased to 80 °C at 10 °C/min, increased to 100 °C at 1 °C/min, then increased to 240 °C at 20 °C/min, and maintained for 2 minutes.

12.2.2 Mass spectrometry/mass spectrometry conditions

a) Ion source temperature: 250 °C.

b) Chromatography and mass spectrometry interface temperature: 250 °C.

ρ – peak area ratio of the N-dimethylnitrosamine chromatographic peak in the sample and the corresponding internal standard chromatographic peak, the corresponding N-dimethylnitrosamine mass concentration calculated from the standard curve, in micrograms per liter (μg/L);

ρ_0 – peak area ratio of the N-dimethylnitrosamine chromatographic peak in the blank test solution and the corresponding internal standard chromatographic peak, the corresponding N-dimethylnitrosamine mass concentration calculated from the standard curve, in micrograms per liter (μg/L);

V – the final constant volume of the test solution, in milliliters (mL);

1 000 – conversion coefficient;

m – sample mass, in grams (g).

Retain 2 digits after the decimal point for the calculation results.

14 Precision

The absolute difference of 2 independent test results obtained under repeatability cannot exceed 20% of the arithmetic mean value.

15 Others

15.1 Method detection limit

When the weighing amount is 5 g, and the constant volume is 10 mL, the detection limit is 0.30 μg/kg, and the quantitation limit is 1.00 μg/kg.

When the weighing amount is 10 g, and the constant volume is 10 mL, the detection limit is 0.15 μg/kg, and the quantitation limit is 0.50 μg/kg.

15.2 Safety warning

The reagents involved in this test that may cause harm to the human body include organic solvents (such as acetonitrile, n-hexane), etc. Test personnel may be at risk due to exposure to these substances. Therefore, test operations involving these substances shall be conducted in a fume hood and professional protective tools (such as gloves, masks, etc.) shall be worn.

Nitrosamine compounds are strong carcinogens. After the test is completed, discarding these substances at will may cause damage to personnel and the environment. At the same time, if handled improperly, the N-dimethylnitrosamine standard used in the test and the expired standard substances may also bring risks. Therefore, these substances

must not be discarded at will during the test; they shall be recovered in closed containers and stored in a well-ventilated environment away from fire sources.

Method 3: Steam distillation-liquid chromatography-mass spectrometry/mass spectrometry

16 Principle

This method uses N-dimethylnitrosamine-D_6 as the internal standard. The internal standard is added to the sample; after steam distillation, the N-dimethylnitrosamine in the sample is absorbed by dichloromethane with the steam and separated by liquid-liquid extraction, which is determined by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and quantified by the internal standard method.

17 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-1 water specified by GB/T 6682.

17.1 Reagents

17.1.1 Dichloromethane (CH_2Cl_2): chromatographic pure.

17.1.2 Concentrated sulfuric acid (H_2SO_4): 18.4 mol/L.

17.1.3 Isooctane (C_8H_{18}): chromatographic pure.

17.1.4 Methanol (CH_4O): chromatographic pure.

17.1.5 Anhydrous sodium sulfate (Na_2SO_4).

17.1.6 Sodium chloride (NaCl): guaranteed reagent.

17.1.7 Formic acid (HCOOH): chromatographic pure.

17.2 Preparation of reagents

17.2.1 Sulfuric acid solution (1+3): Measure 30 mL of concentrated sulfuric acid; slowly pour it into 90 mL of cold water; stir to fully dissipate heat; mix carefully after cooling.

17.2.2 0.1% formic acid aqueous solution: Pipette 1 mL of formic acid into a 1 L volumetric flask; use water to dilute and adjust the volume to the mark; prepare it immediately before use.

17.3 Preparation of standard solutions

corresponding N-dimethylnitrosamine mass concentration calculated from the standard curve, in micrograms per liter (μg/L);

1000 – conversion coefficient;

V – the final constant volume of the test solution, in milliliters (mL);

m – sample mass, in grams (g).

Retain 2 digits after the decimal point for the calculation results.

21 Precision

The absolute difference of 2 independent test results obtained under repeatability cannot exceed 20% of the arithmetic mean value.

22 Others

22.1 Method detection limit

When the weighing amount is 20 g, and the constant volume is 1.0 mL, the detection limit is 0.30 μg/kg, and the quantitation limit is 1.00 μg/kg.

22.2 Safety warning

The reagents involved in this test that may cause harm to the human body include organic solvents (such as acetonitrile, n-hexane), etc. Test personnel may be at risk due to exposure to these substances. Therefore, test operations involving these substances shall be conducted in a fume hood and professional protective tools (such as gloves, masks, etc.) shall be worn.

Nitrosamine compounds are strong carcinogens. After the test is completed, discarding these substances at will may cause damage to personnel and the environment. At the same time, if handled improperly, the N-dimethylnitrosamine standard used in the test and the expired standard substances may also bring risks. Therefore, these substances must not be discarded at will during the test; they shall be recovered in closed containers and stored in a well-ventilated environment away from fire sources.

Method 4: Gas chromatography-thermal energy analysis

23 Principle

The sample is steam distilled, and the N-dimethylnitrosamine in the sample is absorbed by dichloromethane as the vapor, followed by liquid-liquid extraction and separation.

It is measured using a gas chromatography-thermal energy analyzer (GC-TEA) and quantified by the external standard method.

24 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, and the water is grade-1 water specified by GB/T 6682.

24.1 Reagents

Same as 3.1.

24.2 Preparation of reagents

Same as 3.2.

24.3 Standards

Same as 3.3.1.

24.4 Preparation of standard solutions

24.4.1 N-Dimethylnitrosamine standard stock solution (100 μg/mL): Same as 3.4.1.

24.4.2 N-dimethylnitrosamine standard intermediate solution (1 μg/mL): Same as 3.4.2.

24.4.3 N-dimethylnitrosamine standard series working solution: Accurately draw 0.2 mL, 0.5 mL, 1.0 mL, 2.0 mL and 4.0 mL of N-dimethylnitrosamine standard intermediate solution (1 μg/mL), respectively. Place them in the 10 mL volumetric flasks; use dichloromethane to adjust the volume to the mark; mix well. The mass concentrations of N-dimethylnitrosamine standard series working solutions are 20 μg/L, 50 μg/L, 100 μg/L, 200 μg/L and 400 μg/L. Prepare when necessary.

25 Instruments and apparatuses

25.1 Gas chromatograph-thermal energy analyzer (GC-TEA).

25.2 Others are the same as 4.2 ~ 4.10.

26 Analysis steps

26.1 Sample preparation

Except that no internal standard is added, the rest are the same as 5.1.

26.2 Apparatus reference conditions

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