GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard -

Determination of phthalates in food

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Food and Drug Administration.

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Foreword

This standard replaces GB/T 21911-2008 “Determination of phthalate esters in foods” and SN/T 3147-2012 “Determination of phthalates in exported foods”.

As compared with GB/T 21911-2008, the main changes of this standard are as follows:

- CHANGE the standard name to “National food safety standard - Determination of phthalates in food”;
- ADD two target compounds: diallyl phthalate and diisononyl phthalate;
- ADD the isotope internal standard method for quantification as the method I;
- MODIFY the pre-processing method;
- MODIFY the method detection limit.

National food safety standard - Determination of phthalates in food

1 Scope

The first method of this standard specifies the method for the determination of the contents of 16 phthalate esters in food by gas chromatography-mass spectrometry (GC-MS); the second method specifies the method for the determination of the contents of 18 phthalates in foods gas chromatography-mass spectrometry (GC-MS).

The first method of this standard applies to the determination and confirmation, by the internal standard method, of the contents of Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Bis (2-methoxyethyl) phthalate (DMEP), Bis(4-methyl-2-pentyl) phthalate (BMPP), Bis(2-ethoxyethyl) phthalate (DEEP), Dipentyl phthalate (DPP), Dihexyl phthalate (DHXP), Benzylbutyl phthalate (BBP), Bis (2-n-butoxyethyl) phthalate (DBEP), Dicyclohexyl phthalate (DCHP), Bis (2-ethylhexyl) phthalate (DEHP), Diphenylphthalate (DPhP), Di-n-octylphthalate (DNOP) and Dinonyl phthalate (DNP) in foods; the second method is applicable to the determination and confirmation, by the external standard method, of the contents of Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diallyl phthalate (DAP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Bis (2-methoxyethyl) phthalate (DMEP), Bis(4-methyl-2-pentyl) phthalate (BMPP), Bis(2-ethoxyethyl) phthalate (DEEP), Dipentyl phthalate (DPP), Dihexyl phthalate (DHXP), Benzylbutyl phthalate (BBP), Bis (2-n-butoxyethyl) phthalate (DBEP), Dicyclohexyl phthalate (DCHP), Bis (2-ethylhexyl) phthalate (DEHP), Diphenylphthalate (DPhP), Di-n-octylphthalate (DNOP), Diisononyl ortho-phthalate (DINP), Dinonyl phthalate (DNP) in foods.

Method I -- Gas chromatography - Mass spectrometry - Isotope internal standard method

2 Principle

The deuterated phthalate is added to the specimen as an internal standard, various foods are extracted and purified, and then determined by gas chromatography-mass spectrometry. The characteristic selective ion

monitoring scan mode (SIM) is used, the ratio of the retention time to the abundance of the qualitative ion fragments is used for qualitative, the isotope internal standard method is used for quantification.

3 Reagents and materials

Unless otherwise stated, the reagents used in this method are chromatographically pure, the water is the grade II water as specified in GB/T 6682.

3.1 Reagents

3.1.1 n-Hexane (C_6H_{14}).

3.1.2 Acetonitrile (C_2H_3N).

3.1.3 Acetone (CH_3COCH_3).

3.1.4 Dichloromethane (CH_2Cl_2).

3.2 Standard substance

3.2.1 The 16 phthalate standard substances

The mixed liquid standard substance of Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Bis (2-methoxyethyl) phthalate (DMEP), Bis(4-methyl-2-pentyl) phthalate (BMPP), Bis(2-ethoxyethyl) phthalate (DEEP), Dipentyl phthalate (DPP), Dihexyl phthalate (DHXP), Benzylbutyl phthalate (BBP), Bis (2-n-butoxyethyl) phthalate (DBEP), Dicyclohexyl phthalate (DCHP), Bis (2-ethylhexyl) phthalate (DEHP), Di-n-octylphthalate (DNOP) and Dinonyl phthalate (DNP), Diphenylphthalate (DPhP), the concentration is 1000 μg/mL, the standard substance information and purity are as shown in Appendix A.

3.2.2 Phthalate internal standard substance of 16 deuterated isotopes

D_4-Dimethyl phthalate (D_4-DMP), D_4-Diethyl phthalate (D_4-DEP), D_4-Diisobutyl phthalate (D_4-DIBP), D_4-Dibutyl phthalate (D_4-DBP), D_4-Bis (2-methoxyethyl) phthalate (D_4-DMEP), D_4-Bis(4-methyl-2-pentyl) phthalate (D_4-BMPP), D_4-Bis(2-ethoxyethyl) phthalate (D_4-DEEP), D_4-Dipentyl phthalate (D_4-DPP), D_4-Dihexyl phthalate (D_4-DHXP), D_4-Benzylbutyl phthalate (D_4-BBP), D_4-Bis (2-n-butoxyethyl) phthalate (D_4-DBEP), D_4-Dicyclohexyl phthalate (D_4-DCHP), D_4-Bis (2-ethylhexyl) phthalate (D_4-DEHP), D_4-Diphenylphthalate (D_4-DPhP), D_4-Di-n-octylphthalate (D_4-DNOP) and D_4-Dinonyl phthalate (D_4-DNP): purity > 99%.

3.3 Standard solution preparation

3.3.1 The 16 phthalate standard intermediate solutions (10 μg/mL): accurately PIPETTE 1 mL of phthalate standard substance (1000 μg/mL) into a 100 mL volumetric flask, USE the n-hexane to make its volume reach to the mark.

3.3.2 Phthalate internal standard solution of 16 deuterated isotopes (100 μg/mL): Accurately WEIGH 0.01 g of phthalate internal standard of 16 deuterated isotopes (accurate to 0.0001 g) into a 100 mL volumetric flask, USE the n-hexane to make its volume reach to the mark.

3.3.3 Standard use solution of phthalate internal standard of 16 deuterated isotopes (10 μg/mL): Accurately PIPETTE 10 mL of the phthalate internal standard (100 μg/mL) of 16 deuterated isotopes in a 100 mL volumetric flask, ADD n-hexane to make its volume reach to the mark.

3.3.4 The 16 phthalate standard series working solutions: Accurately TAKE 16 kinds of phthalate standard intermediate solution (10 μg/mL), USE n-hexane to gradually dilute it to prepare the standard series solution of concentration 0.00 μg/mL. 0.02 μg/mL, 0.05 μg/mL, 0.10 μg/mL, 0.20 μg/mL, 0.50 μg/mL, 1.00 μg/mL, meanwhile ADD the internal standard use solution (10 μg/mL) to make the concentration of the internal standard of 0.125 μg/mL, PREPARE it before use.

4 Instruments and equipment

Note: After washing the glassware, it is rinsed 3 times with distilled water, it is soaked in acetone for 1 hour, baked at 200 °C for 2 hours, and cooled to room temperature to prepare for use.

4.1 Gas chromatography-Mass spectrometry (GC-MS).

4.2 Analytical balance: Accuracy 0.0001 g.

4.3 Nitrogen blowing instrument.

4.4 Vortex oscillator.

4.5 Ultrasonic generator.

4.6 Centrifuge: Speed ≥ 4000 r/min.

4.7 Crusher.

4.8 Solid phase extraction (SPE) device.

4.9 Solid phase extraction column: PSA/Silica composite packed glass column (1000 mg, 6 mL).

5.2.2.2 Semi-solid specimen B: sesame sauce, oily sauce, etc.

After the sample is fully pulverized and mixed, accurately WEIGH 0.5 g (accurate to 0.0001 g) into a 10 mL stoppered centrifuge tube, ADD 25 μL of isotope internal standard solution, ADD 1 mL of n-hexane, VORTEX it for 2 min, then ADD 5 mL of acetonitrile, VORTEX it for 1 min, PERFORM ultrasonic extraction for 20 min, CENTRIFUGE at 4000 r/min for 5 min, COLLECT the supernatant. ADD 5 mL of acetonitrile, PERFORM extraction again, COMBINE the supernatant. USE 40 °C nitrogen to blow it dry, ADD 6 mL of acetonitrile, VORTEX it uniformly to prepare for SPE purification.

5.2.3 Solid specimen

5.2.3.1 Solid specimen A: milk powder, rice flour, chicken essence, monosodium glutamate, cheese, candy, pollen, meat products, cakes, instant noodles, fruits and vegetables and their products, etc.

Accurately WEIGH 0.5 g (accurate to 0.0001 g) into a 25 mL stoppered centrifuge tube, ADD 125 μL of isotope internal standard solution, ADD 2 mL ~ 5 mL of distilled water, VORTEX it uniformly, then accurately ADD 10 mL of n-hexane, VORTEX it for 1 min, vigorously SHAKE it for 1 min, PERFORM ultrasonic extraction for 30 min, CENTRIFUGE it at 1000 r/min for 5 min, TAKE the supernatant to prepare for GC-MS analysis.

5.2.3.2 Solid specimen B: butter, etc.

After the sample is fully pulverized and mixed, accurately WEIGH 0.5 g (accurate to 0.0001 g) into a 10 mL stoppered centrifuge tube, ADD 25 μL of isotope internal standard solution, ADD 1 mL of n-hexane, VORTEX it for 2 min, then ADD 5 mL of acetonitrile, VORTEX it for 1 min, PERFORM ultrasonic extraction for 20 min, CENTRIFUGE it at 4000 r/min for 5 min, COLLECT the supernatant. ADD 5 mL of acetonitrile to make extraction again, COMBINE the supernatant. USE 40 °C nitrogen to blow it almost dry, ADD 6 mL of acetonitrile, VORTEX it uniformly to prepare for SPE purification.

Note: The butter shall be melted into liquid oil and mixed uniformly before being weighed, it shall be kept in a liquid state during the extraction process.

5.3 SPE purification

ADD 5 mL of dichloromethane and 5 mL of acetonitrile for activation, DISCARD the effluent; ADD the liquid to be purified to the SPE column, COLLECT the effluent; then ADD 5 mL of acetonitrile, COLLECT the effluent, COMBINE the two collected effluent, ADD 1mL of acetone, USE 40 °C nitrogen to blow it almost dry, USE the n-hexane to make its volume reach to 2 mL, VORTEX it uniformly to prepare for GC-MS analysis.

from the standard working curve, in micrograms per milliliter (μg/mL);

V - Constant volume of specimen, in milliliters (mL);

m - The mass of the specimen, in grams (g);

1000 - Conversion factor.

The calculation result shall be deducted from the blank value. When the result is greater than or equal to 1.0 mg/kg, three significant figures are retained; when the result is less than 1.0 mg/kg, two significant figures are retained.

7 Precision

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Other

The limit of quantification of the method is: the limit of quantification of di-n-butyl phthalate (DBP) is 0.3 mg/kg, the limit of quantification of other 15 phthalates except DBP is 0.5 mg/kg.

Method II -- Gas chromatography-mass spectrometry -

External standard method

9 Principle

Various foods are extracted and purified and determined by gas chromatography-mass spectrometry. The characteristic selective ion monitoring scan mode (SIM) is used, the retention time and qualitative ion fragment abundance ratio are used for qualitative, the external standard method is used for quantification.

10 Reagents and materials

Unless otherwise stated, the reagents used in this method are chromatographically pure, the water is grade II water specified in GB/T6682.

10.1 Reagents

Same as 3.1.

Same as 5.1.

12.2 Specimen processing

Except that the isotope internal standard is not added, it is carried out in accordance with the 5.2 determination procedure.

12.3 SPE purification

Same as 5.3.

12.4 Blank test

Except that no specimen is added, the measurement steps are carried out in accordance with 12.2 and 12.3.

12.5 Instrument reference conditions

Except for the scanning method, the rest is same as 5.5.

Scanning method: Selective ion scan (SIM) and monitoring ions are as shown in Appendix D.

12.6 Production of standard curve

Respectively INJECT the standard series working solution into the gas chromatography-mass spectrometer, DETERMINE the chromatographic peak area of the corresponding phthalate, USE the mass concentration of the standard working solution as the abscissa and the corresponding peak area as the ordinate, DRAW a standard curve. The Diisononyl ortho-phthalate standard series working solution are injected for determination separately.

12.7 Determination of specimen solution

The sample solution was injected into a gas chromatography-mass spectrometer to obtain a peak area of the corresponding phthalate, and the concentration of the phthalate in the liquid to be tested was obtained in accordance with a standard curve.

12.8 Qualitative confirmation

Under the conditions of 12.5 instrument, the target compound of the specimen test solution and the phthalate standard appear at the same retention time (±0.5%), and the mass-to-charge ratio of the mass spectrometry fragment ions is consistent with the mass spectrum of the standard, which can be used to qualify the target compounds.

See Appendix E for the total ion chromatogram of phthalates.

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