GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National food safety standard - Determination of acrylamide in food

食品安全国家标准 - 食品中丙烯酰胺的测定

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National food safety standard

Determination of acrylamide in food

1 Scope

This standard specifies the method for determining acrylamide in food.

Method I in this standard, liquid chromatography-mass spectrometry/mass spectrometry, is applicable to the determination of acrylamide in food.

Method II in this standard, gas chromatography-mass spectrometry, is applicable to the determination of acrylamide in foods other than coffee, tea, sugar, infant formula, and complementary foods for infants and young children.

Method I: Liquid chromatography-mass spectrometry/mass spectrometry

2 Principle

Acrylamide in the specimen is extracted with water, purified by matrix dispersion solid phase extraction, solid phase extraction column method or chromatographic column method, and detected by a liquid chromatograph-mass spectrometer/mass spectrometer, with quantification by internal standard method.

3 Reagents and materials

Unless otherwise stated, all reagents used in this method are of analytical grade, and the water is Grade I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Formic acid (HCOOH): chromatographic grade.

3.1.2 Methanol (CH_3OH): chromatographic grade.

3.1.3 Acetonitrile (CH_3CN): chromatographic grade.

3.1.4 *n*-Hexane (*n*-C_6H_{14}).

3.1.5 Ethyl acetate ($CH_3COOC_2H_5$).

3.1.6 Anhydrous sodium sulfate (Na_2SO_4).

3.1.7 Ammonium sulfate [$(NH_4)_2SO_4$].

3.1.8 Sodium chloride (NaCl).

3.1.9 Dry ice (CO_2).

3.2 Reagent preparation

3.2.1 Saturated ammonium sulfate solution: Weigh 80 g of ammonium sulfate crystals, add 100 mL of water, sonicate to dissolve, and let stand at room temperature. Prepare fresh before use.

3.2.2 Formic acid-water solution (0.1%): Accurately transfer 1 mL of formic acid, dilute with water to 1000 mL, and mix thoroughly. Prepare fresh before use.

3.3 Standard substances

3.3.1 Acrylamide (C_3H_5NO, CAS No.: 79-06-1) standard: with a purity of ≥99%, or a standard substance that has been certified by the nation and awarded a certificate of reference material.

3.3.2 $^{13}C_3$-Acrylamide ($^{13}C_3H_5NO$, CAS No.: 287399-26-2) standard: with a purity of ≥98%.

3.4 Preparation of standard solutions

3.4.1 Preparation of acrylamide standard solution

3.4.1.1 Acrylamide standard stock solution (1000 mg/L): Accurately weigh 10.0 mg of acrylamide standard (accurate to 0.01 mg), dissolve in methanol and dilute to 10 mL, mix well to achieve an acrylamide mass concentration of 1000 mg/L. Transfer the solution to a tightly sealed brown glass container and store at -18 °C protected from light. Shelf life is 6 months.

3.4.1.2 Acrylamide intermediate solution (100 mg/L): Transfer 1 mL of acrylamide standard stock solution (1000 mg/L) to a 10 mL volumetric flask, dilute to the mark with methanol, and mix well to achieve an acrylamide mass concentration of 100 mg/L. Transfer the solution to a tightly sealed brown glass container and store at -18 °C in the dark. Shelf life is 3 months.

3.4.1.3 Acrylamide working solution I (10 mg/L): Transfer 1 mL of acrylamide intermediate solution (100 mg/L) to a 10 mL volumetric flask, dilute with water and bring to the mark, mix well to achieve an acrylamide mass concentration of 10 mg/L.

Prepare fresh before use.

3.4.1.4 Acrylamide working solution II (1 mg/L): Transfer 1 mL of acrylamide working solution I (10 mg/L) to a 10 mL volumetric flask, dilute with water and bring to the mark, mix well to achieve an acrylamide mass concentration of 1 mg/L. Prepare fresh before use.

3.4.2 Preparation of $^{13}C_3$-acrylamide internal standard solution

3.4.2.1 $^{13}C_3$-Acrylamide stock solution (1000 mg/L): Accurately weigh 10.0 mg of $^{13}C_3$-acrylamide standard (accurate to 0.01 mg), dissolve in methanol and dilute to 10 mL, mix well to achieve a $^{13}C_3$-acrylamide mass concentration of 1000 mg/L. Transfer the solution to a tightly sealed brown glass container and store at -18 °C protected from light. Shelf life is 6 months.

3.4.2.2 $^{13}C_3$-Acrylamide working solution (10 mg/L): Transfer 1 mL of $^{13}C_3$-acrylamide internal standard stock solution (1000 mg/L) to a 100 mL volumetric flask, dilute with water and bring to the mark, mix well, and prepare the $^{13}C_3$-acrylamide internal standard solution to a mass concentration of 10 mg/L. Prepare fresh before use.

3.4.3 Standard series working solution

Transfer 0.10 mL, 0.50 mL, and 1.00 mL of acrylamide working solution II (1 mg/L) and 0.20 mL, 0.50 mL, and 1.00 mL of acrylamide working solution I (10 mg/L) to 10 mL volumetric flasks, respectively. Add 0.10 mL of $^{13}C_3$-acrylamide internal standard working solution (10 mg/L), dilute to the mark with water, and mix well. The mass concentrations of acrylamide in the standard series solutions are 10 μg/L, 50 μg/L, 100 μg/L, 200 μg/L, 500 μg/L, and 1000 μg/L, respectively, and the internal standard concentration of $^{13}C_3$-acrylamide is 100 μg/L. The concentrations of the standard series working solutions can be adjusted appropriately according to the concentration of the actual sample solution. Prepare fresh before use.

3.5 Materials

3.5.1 Granular diatomaceous earth: Extrelut™ 20 or an equivalent product.

3.5.2 Matrix dispersion purification tube: It contains 20 mg of graphitized carbon black, 150 mg of octadecyl adsorbent, 150 mg of ethylenediamine-*N*-propylsilane, 200 mg of strong cation exchange material and 150 mg of magnesium sulfate; or equivalent products.

3.5.3 Hydrophilic-lipophilic balanced solid phase extraction column: The packing material is a copolymer of divinylbenzene and *N*-vinylpyrrolidone, the column capacity is 6 mL, and the packing material amount is 200 mg; or an equivalent product.

3.5.4 Mixed silica bonded solid phase extraction column: The packing material is a

mixed bond of strong cation exchange and strong anion exchange, with a column capacity of 3 mL and a packing material amount of 200 mg; or an equivalent product.

3.5.5 Glass chromatographic column: with a length of 30 cm and an inner diameter of 1.8 cm.

3.5.6 Aqueous microporous filter membranes: 0.22 μm and 0.45 μm.

4 Instruments and equipment

4.1 Liquid chromatograph-mass spectrometer/mass spectrometer (LC-MS/MS), with an electrospray ionization source.

4.2 Tissue grinder.

4.3 Rotary evaporator.

4.4 Nitrogen blower.

4.5 Oscillator.

4.6 Vortex mixer.

4.7 Electronic balance: with a sensitivity of 0.01 mg and 1 mg.

4.8 Centrifuge: with a speed of ≥10000 r/min.

5 Analysis steps

5.1 Specimen preparation

5.1.1 Baked goods, puffed foods, and fried cereal foods

Take 100 g of the specimen, add 100 g of dry ice, and then grind it evenly using a tissue grinder. After the dry ice has completely evaporated, store it frozen at -18 ℃.

NOTE: Dry ice has an extremely low temperature. Thick cotton gloves or other protective coverings (such as clamps) shall be used to handle dry ice. When dry ice sublimates, it releases carbon dioxide, which causes the internal pressure of the tissue grinder to rise. Attention shall be paid to its pressure resistance to prevent the chamber from bursting due to excessive pressure. Good ventilation shall also be ensured.

5.1.2 Coffee, tea, sugar, infant formula and complementary foods for infants and young children

Take 100 g of the specimen, grind it evenly with a tissue grinder (instant coffee and

infant formula are sampled directly), and store it frozen at -18 ℃.

NOTE: During the pulverization process, prevent the sample from being overheated, which could affect the measurement results.

5.2 Specimen extraction

Accurately weigh 0.5 g~2 g of the specimen (accurate to 0.001 g), add 10 μL (by column chromatography) or 20 μL (by matrix dispersion solid-phase extraction and solid-phase extraction column methods) of 10 mg/L $^{13}C_3$-acrylamide internal standard working solution, then add 10 mL of water, shake for 30 min, centrifuge at 4000 r/min for 10 min, and collect the supernatant for purification. If the supernatant cannot be obtained by centrifugation, such as for matrix samples like instant coffee and infant formula, collect the suspension for purification.

5.3 Specimen purification

5.3.1 Matrix dispersion solid phase extraction (applicable to all foods)

Add 5 mL of *n*-hexane to the supernatant of the specimen extraction, shake and extract for 5 min, centrifuge at 10000 r/min for 5 min to remove the organic phase, and repeat the extraction once more with 5 mL of *n*-hexane. Add 10 mL of acetonitrile to the extract, shake and extract for 10 min, add 0.5 g of sodium chloride and 4 g of anhydrous sodium sulfate as salting-out agents, and shake for 5 min. Centrifuge at 10000 r/min for 5 min, take 2.00 mL of the supernatant into a matrix dispersion purification tube, vortex for 5 min, and centrifuge at 10000 r/min for 5 min; take 1 mL of the supernatant and concentrate to near dryness in a nitrogen blower, redissolve with 0.20 mL of water, filter through a 0.22 μm aqueous filter membrane for later analysis.

5.3.2 Other purification methods (applicable to foods other than coffee, tea, sugar, infant formula, and complementary foods for infants and young children)

5.3.2.1 Solid-phase extraction column method

Add 5 mL of *n*-hexane to the supernatant of the specimen extraction, shake and extract for 10 min, centrifuge at 10000 r/min for 5 min to remove the organic phase, and repeat the extraction once more with 5 mL of *n*-hexane. Take 6 mL of the extract and filter it through a 0.45 μm aqueous filter membrane. Collect the filtrate for purification using a hydrophilic-lipophilic equilibrium solid-phase extraction column. Before use, the hydrophilic-lipophilic equilibrium solid-phase extraction column is activated sequentially with 3 mL of methanol and 3 mL of water. Add 5.00 mL of the filtrate to a hydrophilic-lipophilic balanced solid-phase extraction column, collect the effluent, and elute with 4 mL of 80% methanol aqueous solution. Collect all the eluent and combine it with the effluent for purification using a mixed-bonded solid-phase extraction column. Activate the mixed-bonded solid-phase extraction column sequentially with 3 mL of

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