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GB 5009.204-2025

Replacing GB 5009.204-2014

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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Standardization Administration of the PRC

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1 Scope

1 The document lays down the methods for determining acrylamide in food.

1 The first method, liquid chromatography-tandem mass spectrometry, applies to the determination of acrylamide in food.

1 The second method, gas chromatography-mass spectrometry, applies to the determination of acrylamide in food other than coffee, tea, sugar, infant formula food and complementary food for infants and young children.

2 Principle of the first method

2 The acrylamide in the test sample is extracted with water, cleaned up by dispersive solid phase extraction, by a solid phase extraction cartridge or by a packed chromatographic

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column, and measured by liquid chromatography-tandem mass spectrometry with quantification by the internal standard method.

3 Reagents and materials of the first method

3 Unless otherwise stated the reagents are of analytical grade and the water is grade one water as defined in GB/T 6682.

3.1 The reagents are formic acid, methanol and acetonitrile of chromatographic grade, together with n-hexane, ethyl acetate, anhydrous sodium sulfate, ammonium sulfate, sodium chloride and dry ice.

3.2 A saturated ammonium sulfate solution and a 0.1% formic acid solution in water are prepared, both immediately before use.

3.3 The standards are acrylamide, CAS number 79-06-1, of purity not less than 99%, or a nationally certified standard issued with a reference material certificate, and carbon-13 labelled acrylamide, CAS number 287399-26-2, of purity not less than 98%.

3.4 An acrylamide stock standard solution of 1000 mg/L in methanol is prepared and kept at -18 °C protected from light for up to six months; an intermediate solution of 100 mg/L in methanol is valid for three months; working solutions I and II of 10 mg/L and 1 mg/L in water are prepared immediately before use. The internal standard stock solution of 1000 mg/L in methanol is likewise valid for six months and its working solution of 10 mg/L in water is prepared immediately before use. The series of working standard solutions is made up in water from working solutions I and II with the addition of the internal standard working solution, giving acrylamide concentrations of 10, 50, 100, 200, 500 and 1000 micrograms per litre and an internal standard concentration of 100 micrograms per litre; the concentrations may be adjusted to suit the samples.

3.5 The materials are granular diatomaceous earth; a dispersive clean-up tube containing graphitised carbon black, an octadecyl sorbent, ethylenediamine-N-propylsilane, a strong cation exchange material and magnesium sulfate, or an equivalent; a hydrophilic-lipophilic balanced solid phase extraction cartridge of 6 mL capacity holding 200 mg of a divinylbenzene and N-vinylpyrrolidone copolymer, or an equivalent; a mixed-mode silica bonded cartridge of 3 mL capacity holding 200 mg of a mixed strong cation and strong anion exchange bonded phase, or an equivalent; a glass chromatographic column 30 cm long and 1.8 cm in internal diameter; and aqueous microporous filter membranes of 0.22 micrometre and 0.45 micrometre pore size.

4 Instruments and equipment of the first method

4 The equipment is a liquid chromatograph-tandem mass spectrometer fitted with an electrospray ionisation source; a tissue grinder; a rotary evaporator; a nitrogen evaporator; a shaker; a vortex mixer; an electronic balance with graduations of 0.01 mg and 1 mg; and

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a centrifuge reaching at least 10000 r/min.

5 Analytical procedure of the first method

5.1 For baked foods, puffed foods and fried cereal foods, 100 g of sample is ground with 100 g of dry ice in a tissue grinder and stored at -18 °C once the dry ice has fully sublimed. A note warns that dry ice is extremely cold and is to be handled with thick cotton gloves or tongs, that the carbon dioxide released as it sublimes raises the pressure inside the grinder, so that the pressure rating of the chamber is to be respected to prevent it bursting, and that the room is to be well ventilated. For coffee, tea, sugar, infant formula food and complementary food for infants and young children, 100 g of sample is ground in a tissue grinder, instant coffee and infant milk powder being sampled directly, and stored at -18 °C; a note asks that overheating during grinding be avoided.

5.2 For extraction, 0.5 g to 2 g of sample is weighed to the nearest 0.001 g, the internal standard working solution is added, 10 microlitres for the packed column method and 20 microlitres for the dispersive and cartridge methods, and 10 mL of water is added. The mixture is shaken for 30 min and centrifuged at 4000 r/min for 10 min, and the supernatant is taken for clean-up. Where no supernatant can be obtained by centrifugation, as with instant coffee or infant milk powder, the suspension is taken instead.

5.3.1 In the dispersive solid phase extraction clean-up, which applies to all foods, the supernatant is extracted twice with 5 mL of n-hexane with centrifugation at 10000 r/min, the organic phase being discarded each time; 10 mL of acetonitrile is added and the mixture is extracted for 10 min, 0.5 g of sodium chloride and 4 g of anhydrous sodium sulfate are added as salting-out agents and the mixture is shaken for 5 min and centrifuged; 2.00 mL of the supernatant is vortexed for 5 min with the dispersive clean-up tube and centrifuged, and 1 mL of the resulting supernatant is concentrated almost to dryness under nitrogen, redissolved in 0.20 mL of water and filtered through a 0.22 micrometre aqueous membrane before measurement.

5.3.2 The other clean-up methods apply to foods other than coffee, tea, sugar, infant formula food and complementary food for infants and young children. In the cartridge method the supernatant is extracted twice with n-hexane, filtered through a 0.45 micrometre membrane, passed through a hydrophilic-lipophilic balanced cartridge conditioned with methanol and water and eluted with 4 mL of 80% aqueous methanol, and the combined effluent and eluate are passed under gravity through a mixed-mode bonded cartridge likewise conditioned with methanol and water; the whole effluent is concentrated almost to dryness under nitrogen, made up to 1.00 mL with water and filtered through a 0.22 micrometre membrane. In the packed column method 15 g of ammonium sulfate is dissolved in the supernatant, the mixture is shaken for 10 min and centrifuged, and 10 mL of the supernatant, made up if necessary with saturated ammonium sulfate solution, is mixed with 5 g of diatomaceous earth and charged onto a glass column packed with glass

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wool, 10 g of anhydrous sodium sulfate and 2 g of diatomaceous earth; the column is washed with 70 mL of n-hexane, which is discarded, and eluted with 70 mL of ethyl acetate at a flow rate of 2 mL/min, and the eluate is evaporated almost to dryness under reduced pressure in a water bath at 45 °C, the residue being washed over with three 1 mL portions of ethyl acetate into a tube containing 1.00 mL of water; after the organic layer has been blown off under nitrogen, 1 mL of n-hexane is added, the tube is vortexed and centrifuged, and the lower aqueous phase is filtered through a 0.22 micrometre membrane before measurement.

5.4 The reference chromatographic conditions are a C18 column of 3.0 mm by 150 mm and 1.8 micrometre particle size, with a stated carbon load of 9%, a pore size of 9.5 nm, double end-capping and a specific surface area of 160 m2/g, or an equivalent, with a matching guard column; mobile phase A of 0.1% formic acid in water and mobile phase B of methanol, run under the gradient of Table 1; a flow rate of 0.2 mL/min; an injection volume of 10 microlitres; and ambient column temperature. The gradient of Table 1 holds 98% A and 2% B from 0.0 min to 2.0 min, changes to 90% A and 10% B by 4.0 min and holds to 6.0 min, changes to 5% A and 95% B by 7.0 min and holds to 8.5 min, and returns to 98% A and 2% B by 8.6 min, held to 10.0 min.

5.4.2 The reference mass spectrometric conditions are multiple reaction monitoring; positive electrospray ionisation; a capillary voltage of 2500 V; an ion source temperature of 150 °C; a desolvation gas temperature of 600 °C; a cone gas flow of 150 L/h; a nebuliser gas pressure of 700 kPa; and a collision gas flow of 0.15 mL/min. The ion pairs, declustering voltages and collision energies are given in Table 2, whose rows the extracted text runs together so that the individual figures cannot be assigned to their columns; the quantifier transitions are named in the body of the document as m/z 72 greater than 55 for acrylamide and m/z 72 greater than 58 for the carbon-13 labelled internal standard.

5.5 The calibration curve is drawn by injecting the series of working standard solutions and plotting the ratio of the peak areas of acrylamide and of its internal standard, taken from the quantifier ion mass chromatograms, against the acrylamide concentration in micrograms per litre. The reference mass chromatogram is Figure A.1 of Annex A.

5.6 For qualitative determination, the retention time of the analyte peak in the sample is to agree with that in the corresponding standard solution to within plus or minus 2.5%, both the quantifier and the qualifier ions are to appear, and the relative abundance ratio of the qualifier to the quantifier ion is to agree with that of a standard solution of comparable concentration within the deviations of Table 3, namely plus or minus 20% where the relative abundance is greater than 50%, plus or minus 25% where it is greater than 20% and not greater than 50%, plus or minus 30% where it is greater than 10% and not greater than 20%, and plus or minus 50% where it is not greater than 10%.

5.7 For quantitative determination the ratio of the peak areas of acrylamide at m/z 72 greater than 55 and of the internal standard at m/z 72 greater than 58 is measured and the

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