NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 5009.304-2025

National food safety standard - Determination of trivalent chromium and hexavalent chromium in foods

食品安全国家标准 食品中三价铬和六价铬的测定

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2 Principle (part one)

The test portion is extracted with an alkaline solution by shaking, centrifuged at high speed and measured on a liquid chromatograph coupled to an inductively coupled plasma mass spectrometer.

3 Reagents and materials (part one)

Unless otherwise stated the reagents are of analytical grade and the water is grade one water as specified in GB/T 6682. The reagents listed are nitric acid of concentration not less than 69 percent, ammonia solution of concentration not less than 25 percent, sodium hydroxide, helium of purity not less than 99.995 percent, and argon of purity not less than 99.995 percent or liquid argon.

3.2 The prepared solutions are a 60 mmol/L ammonium nitrate solution, made by taking 3.9 mL of nitric acid into 1 L of water and adjusting the pH to 7.0 +/- 0.1 with ammonia solution, and a 0.05 mol/L sodium hydroxide solution, made by weighing 1 g of sodium hydroxide, dissolving it in water and making up to 500 mL.

3.3 to 3.4 The standard is a hexavalent chromium standard solution at 1000 μg/mL in a water medium, being a certified reference material issued with a national certificate. The hexavalent chromium standard stock solution at 10 μg/mL is made by taking 1.0 mL of that standard solution into a 100 mL volumetric flask, making up to the mark with water, mixing, transferring to a brown reagent bottle and keeping it at 4 °C in the dark, where it is valid for 1 month. The hexavalent chromium working standard solution at 100 μg/L is made by taking 1.0 mL of the stock solution into a 100 mL volumetric flask, making up to the mark with the 60 mmol/L ammonium nitrate solution, mixing and transferring to a brown reagent bottle; it is made up freshly before use.

4 Apparatus and equipment (part one)

The apparatus comprises a liquid chromatograph-inductively coupled plasma mass spectrometer, made up of a liquid chromatograph and an inductively coupled plasma mass spectrometer fitted with a collision reaction cell; a balance with a readability of 0.001 g; a pH meter accurate to 0.01; a water bath shaker; a refrigerated centrifuge with a centrifugal force of not less than 13100 g; and an aqueous syringe filter with a 0.45 μm mixed cellulose membrane.

5 Analytical procedure (part one)

5.1 Contamination of the sample shall be prevented during preparation and storage. Powdered and liquid samples are mixed until uniform, placed in clean containers, sealed, labelled and held at 4 °C until required.

5.2 A test portion of 0.25 g is accurately weighed to the nearest 0.001 g into a stoppered polypropylene centrifuge tube, 5 mL of water is added and the sample dispersed by vortexing, then 20 mL of the 0.05 mol/L sodium hydroxide solution is added and the tube is shaken in a water bath at 25 °C for 0.5 h. The tube is centrifuged at 4 °C at 10000 r/min for 10 min, and the upper clear layer is passed through a 0.45 μm membrane for measurement on the liquid chromatograph-inductively coupled plasma mass spectrometer. A blank test is carried out at the same time.

5.3 The reference chromatographic conditions are a Dionex AG19 guard column of 4.1 mm x 50 mm packed with 10 μm material together with an AS19 analytical column of 4.1 mm x 250 mm packed with 10 μm material, or anion exchange columns of equivalent performance; a column temperature of 25 °C; a mobile phase of 60 mmol/L ammonium nitrate at pH 7.0; a flow rate of 1.0 mL/min; and an injection volume of 50 μL. The mass

spectrometric conditions are given in Annex A.

5.4 to 5.5 Volumes of 0 μL, 5 μL, 10 μL, 20 μL, 50 μL, 100 μL and 200 μL of the hexavalent chromium working standard solution are taken and made up to 1 mL with water, giving a series of standard solutions at hexavalent chromium mass concentrations of 0 ng/mL, 0.5 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, 10.0 ng/mL and 20.0 ng/mL. These are measured on the liquid chromatograph-inductively coupled plasma mass spectrometer and the working curve is drawn with the peak area of hexavalent chromium on the vertical axis and its concentration on the horizontal axis. Under the same conditions the blank solution and the sample solution are injected and measured, and the concentration of hexavalent chromium in the test solution is calculated from the working curve.

6 Expression of results (part one)

The hexavalent chromium content of the test portion is calculated by Formula (1), whose symbols are: x1, the hexavalent chromium content of the test portion, in milligrams per kilogram; rho, the mass concentration of hexavalent chromium in the test solution taken from the working curve, in nanograms per millilitre; rho zero, the mass concentration of hexavalent chromium in the blank test solution, in nanograms per millilitre; V, the volume of the extraction solution, in millilitres; and m, the mass of the test portion, in grams. The result is reported to three significant figures.

7 Precision (part one)

The absolute difference between two independent results obtained under repeatability conditions shall not exceed 15 percent of their arithmetic mean.

8 Other (part one)

With a test portion of 0.25 g and an extraction solution volume of 25 mL, the detection limit of the method for hexavalent chromium is 0.02 mg/kg and the quantification limit is 0.05 mg/kg.

9 Principle (part two)

The test portion is extracted with nitric acid solution to bring trivalent chromium into solution, sodium thiosulfate solution is added so that trivalent chromium is not converted into hexavalent chromium, the pH is adjusted to alkaline, the analyte is complexed with disodium ethylenediaminetetraacetate solution, and after high speed centrifugation the trivalent chromium is measured on the liquid chromatograph-inductively coupled plasma mass spectrometer. Under these conditions trivalent and hexavalent chromium convert in equal amounts, so the amount of hexavalent chromium determined in the first part is subtracted from the measured amount of trivalent chromium to give the trivalent chromium

content of the sample.

10 Reagents and materials (part two)

Unless otherwise stated the reagents are of analytical grade and the water is grade one water as specified in GB/T 6682. The reagents listed are sodium thiosulfate pentahydrate, disodium ethylenediaminetetraacetate, nitric acid of ultra-high purity and concentration not less than 69 percent, ammonia solution of chromatographic grade and concentration not less than 25 percent, helium of purity not less than 99.995 percent, and argon of purity not less than 99.995 percent or liquid argon.

10.2 The prepared solutions are a 0.1 mol/L sodium thiosulfate solution, made by weighing 26 g of sodium thiosulfate and dissolving it in 1000 mL of boiled and cooled water; a 60 mmol/L ammonium nitrate solution, made by taking 3.9 mL of nitric acid into 1 L of water and adjusting the pH to 7.0 +/- 0.1 with ammonia solution; a 20 mmol/L disodium ethylenediaminetetraacetate solution, made by weighing 3.38 g of the salt, dissolving it in the 60 mmol/L ammonium nitrate solution, adding water to almost 500 mL, adjusting the pH to 9.0 +/- 0.1 with ammonia solution and making up to 500 mL in a volumetric flask with water; and a 5 percent nitric acid solution, made by weighing an amount of nitric acid equivalent to 5 g into a 100 mL volumetric flask and diluting to the mark with water.

10.3 to 10.4 The standard is a trivalent chromium standard solution at 1000 μg/mL in a hydrochloric acid medium, being a certified reference material issued with a national certificate. The trivalent chromium standard stock solution at 10 μg/mL is made by taking 1.0 mL of that standard solution into a 100 mL volumetric flask, making up to the mark with hydrochloric acid, mixing, transferring to a brown reagent bottle and keeping it at 4 °C in the dark, where it is valid for 1 month. The trivalent chromium working standard solution at 1000 μg/L is made by taking 10.0 mL of the stock solution into a 100 mL volumetric flask, making up to the mark with the 60 mmol/L ammonium nitrate solution, mixing and transferring to a brown reagent bottle; it is made up freshly before use.

11 Apparatus and equipment (part two)

The apparatus is the same as that of Clause 4.

12 Analytical procedure (part two)

12.1 Sample preparation is the same as in 5.1.

12.2 A test portion of 0.5 g is accurately weighed to the nearest 0.001 g into a stoppered polypropylene centrifuge tube, 5 mL of the 5 percent nitric acid solution is added and the tube is shaken in a water bath at 80 °C for 1 h. After cooling, 5 mL of the 0.1 mol/L sodium thiosulfate solution is added, the pH is adjusted to 9.0 +/- 0.1 with ammonia solution, 15 mL of the 20 mmol/L disodium ethylenediaminetetraacetate solution is added and the tube is