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National Food Safety Standard - Determination of Sucralose in Foods

食品安全国家标准　食品中三氯蔗糖（蔗糖素）的测定

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Table of Contents

National Food Safety Standard - Determination of Sucralose in Foods

1 Scope

This Standard specifies the high performance liquid chromatography and high performance liquid chromatography - tandem mass spectrometry for the determination of sucralose in foods.

This Standard is applicable to the determination of sucralose in foods.

Method I - High Performance Liquid Chromatography

2 Principle

The sucralose in the specimen is extracted with methanol-water solution to remove protein and fat. After purification by a solid phase extraction column, evaporation to dryness, redissolution and enrichment, a high-performance liquid chromatograph is used, and a reversed-phase C_{18} chromatographic column is used for separation. A differential detector or an evaporative light-scattering detector is adopted for detection. In accordance with the retention time of the chromatographic peak, conduct qualitative determination. Adopt the external standard method for quantitative determination.

3 Reagents and Materials

Unless it is otherwise specified, the reagents used in this Method are all analytically pure, and the water is Grade-1 water specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_3OH).

3.1.2 Methanol (CH_3OH): chromatographically pure.

3.1.3 n-hexane (C_6H_{14}).

3.1.4 Dipotassium hydrogen phosphate ($K_2HPO_4 \bullet 3H_2O$).

3.1.5 Zinc acetate [$Zn(CH_3COO)_2 \bullet 2H_2O$].

3.1.6 Potassium ferrocyanide [$K_4Fe(CN)_6 \bullet 3H_2O$].

3.1.7 Acetic acid (CH_3COOH).

3.1.8 Acetonitrile (CH_3CN): chromatographically pure.

3.1.9 Neutral alumina: with a particle size of 75 μm ~ 150 μm (100 mesh ~ 200 mesh).

3.2 Preparation of Reagents

3.2.1 Zinc acetate solution (219 g/L): weigh-take 21.9 g of zinc acetate, add 3 mL of acetic acid, and add water to dissolve it to 100 mL.

3.2.2 Potassium ferrocyanide solution (106 g/L): weigh-take 10.6 g of potassium ferrocyanide and add water to dissolve it to 100 mL.

3.2.3 Dipotassium hydrogen phosphate solution (0.1%): weigh-take 0.1 g of dipotassium hydrogen phosphate and add water to dissolve it to 100 mL.

3.2.4 Methanol-0.1% dipotassium hydrogen phosphate solution (20 + 80): evenly mix methanol and 0.1% dipotassium hydrogen phosphate solution in a volume ratio of 20 : 80.

3.2.5 Methanol-water solution (75 + 25): evenly mix methanol and water in a volume ratio of 75 : 25.

3.2.6 Water-acetonitrile solution (89 + 11): evenly mix water and acetonitrile in a volume ratio of 89 : 11.

3.3 Reference Material

Sucralose reference material ($C_{12}H_{19}Cl_3O_8$, CAS: 56038-13-2): purity ≥ 99%, or a standard substance certified by the state and awarded a reference material certificate.

3.4 Preparation of Standard Solutions

3.4.1 Sucralose standard stock solution (10.0 mg/mL): weigh-take 0.25 g (accurate to 0.0001 g) of sucralose reference material, use water to dissolve it and transfer to a 25 mL volumetric flask. Reach a constant volume to the scale and evenly mix it. The stock solution is stored in a 4 °C refrigerator with a shelf life of 6 months.

3.4.2 Sucralose standard intermediate solution (1.00 mg/mL): transfer-take 5.00 mL of sucralose standard stock solution (10.0 mg/mL) in a 50 mL volumetric flask, use water to reach a constant volume to the scale and evenly mix it. Store it in a 4 °C refrigerator with a shelf life of 3 months.

3.4.3 Sucralose standard series of working solutions: transfer-take an appropriate amount of sucralose standard intermediate solution (1.00 mg/mL), use water to dilute it and prepare standard series of working solutions with a mass concentration of 0.0200 mg/mL, 0.0500 mg/mL, 0.100 mg/mL, 0.200 mg/mL, 0.400 mg/mL, 0.800 mg/mL and 1.00 mg/mL, respectively. Prepare them right before use.

3.5 Materials

5.2.1.2 Alcohol specimens containing solid substance

Weigh-take 2 g ~ 5 g (accurate to 0.001 g) of evenly mixed alcohol specimen in an evaporating dish, heat it on a 60 °C water bath for 30 minutes, use 10 mL of water to rinse the residue in the evaporating dish in three times, and combine the washing fluids and transfer them to a 15 mL centrifuge tube. On a vortex mixer, oscillate it for 3 minutes, perform ultrasonic extraction for 20 minutes, then, at 8,500 r/min, centrifuge for 5 minutes. Use 5 mL of water to repeat the extraction once, combine the extracting solutions and reserve the supernatant for purification.

5.2.2 Vinegar, soy sauce, sauce and sauce products specimens

5.2.2.1 Weigh-take 2 g ~ 5 g (accurate to 0.001 g) of evenly mixed specimen in a 50 mL centrifuge tube, add 1.0 g of neutral alumina and add 5 mL of water. On a vortex mixer, oscillate it for 3 minutes, then, add 15 mL of methanol, and continue to oscillate for 30 s, perform ultrasonic extraction for 20 minutes; at 3,000 r/min, centrifuge for 10 minutes, and transfer the supernatant into the 50 mL centrifuge tube. Add the precipitate to 5.0 mL of methanol-water solution (75 + 25), use a glass rod to evenly stir it, then, oscillate it on a vortex mixer for 30 s. At 3,000 r/min, centrifuge for 10 min, repeat the extraction twice, and combine the supernatants.

5.2.2.2 Transfer all the supernatants to a separatory funnel, add 30 mL of n-hexane, shake it for 2 minutes, and let it stand for 20 minutes for stratification. Transfer the lower aqueous phase to an evaporating dish and evaporate it on the boiling water bath. When the liquid in the evaporating dish is about 1 mL, use 9 mL of water to rinse the evaporating dish in three times, and combine the washing fluids and transfer them to a 15 mL centrifuge tube. Conduct ultrasonication for 5 minutes; at 3,000 r/min, centrifuge for 10 minutes and reserve it for purification.

5.2.3 Jelly, candies and candied fruits specimens

Weigh-take 2 g ~ 5 g (accurate to 0.001 g) of pulverized and evenly mixed specimen in a 50 mL centrifuge tube and add 5 mL of water. On a vortex mixer, oscillate it for 3 minutes, then, add 15 mL of methanol, 0.50 mL of zinc acetate solution and 0.50 mL of potassium ferrocyanide solution, continue the oscillation for 30 s. Then, in a 60 °C water bath, heat it for 15 minutes. During the water bath, shake and disperse it, and the following steps shall be successively handled, starting from “perform ultrasonic extraction for 20 minutes; at 3,000 r/min, centrifuge for 10 minutes” in 5.2.2.1, till “conduct ultrasonication for 5 minutes; at 3,000 r/min, centrifuge for 10 minutes and reserve it for purification” in 5.2.2.2.

5.2.4 Other specimens

Weigh-take 2 g ~ 5 g (accurate to 0.001 g) of evenly mixed specimen into a 50 mL centrifuge tube and add 5 mL of water. On a vortex mixer, oscillate for 3 minutes, then add 15 mL of methanol, 0.50 mL of zinc acetate solution and 0.50 mL of potassium ferrocyanide solution. The following steps shall be successively handled, starting from “continue to oscillate for 30 s, perform ultrasonic extraction for 20 minutes; at 3,000 r/min, centrifuge for 10 minutes” in 5.2.2.1, till “conduct ultrasonication for 5 minutes; at 3,000 r/min, centrifuge for 10 minutes and reserve it for purification” in 5.2.2.2.

5.3 Specimen Purification

5.3.1 Differential detector

Before use, the solid phase extraction column is successively activated with 4 mL of methanol and 4 mL of water to maintain the column moist.

Transfer all the specimen extraction supernatants into the activated solid phase extraction column and control the liquid flow rate to no more than 1 drop/s. When the liquid level on the column is about 2 mm, add 1 mL of water, and continue to maintain the liquid flow rate at 1 drop/s. After the liquid in the column is completely discharged, use 3 mL of methanol to elute it, collect all the eluent and evaporate it to dryness on a boiling water bath. Use 1.00 mL of water to dissolve the residue (if the solution is turbid, it can be transferred into a centrifuge tube, at 10,000 r/min, centrifuge for 5 minutes), filter it through a 0.45 μm hydrophilic microporous filter membrane. The filtrate is the prepared specimen solution to be tested.

5.3.2 Evaporative light-scattering detector

Before use, the solid phase extraction column is successively activated with 4 mL of methanol and 4 mL of water to maintain the column moist.

Transfer all the specimen extraction supernatants into the activated solid phase extraction column and control the liquid flow rate to no more than 1 drop/s. When the liquid level on the column is about 2 mm, add 1 mL of water, and continue to maintain the liquid flow rate at 1 drop/s. After the liquid in the column is completely discharged, use 3 mL of methanol to elute it, collect all the eluent and evaporate it to dryness on a boiling water bath. Use 1.00 mL of water-acetonitrile solution (89 + 11) to dissolve the residue (if the solution is turbid, it can be transferred into a centrifuge tube, at 10,000 r/min, centrifuge for 5 minutes), filter it through a 0.45 μm hydrophobic microporous filter membrane. The filtrate is the prepared specimen solution to be tested.

NOTE: the supernatant of jelly samples after extraction needs to be heated in a 50 °C water bath, then, pass through the column while it is still hot, otherwise, the extraction column will be easily blocked.

5.4 Blank Test

The pre-treatment of different specimens requires a blank test of the specimens to be simultaneously conducted.

5.5 Reference Conditions of Instruments

5.5.1 Differential detector

5.5.1.1 Chromatographic column: C_{18} (250 mm × 4.6 mm, 5 μm), or equivalent column.

5.5.1.2 Mobile phase: methanol-0.1% dipotassium hydrogen phosphate solution (20 + 80).

5.5.1.3 Flow rate: 1.0 mL/min.

5.5.1.4 Column temperature: 35 °C.

5.5.1.5 Detection cell temperature: 35 °C.

5.5.1.6 Sensitivity: 16.

5.5.1.7 Injection volume: 20 μL.

5.5.2 Evaporative light-scattering detector

5.5.2.1 Chromatographic column: C_{18} (250 mm × 4.6 mm, 5 μm), or equivalent column.

5.5.2.2 Mobile phase: A is water, B is acetonitrile, water + acetonitrile = 89 + 11.

NOTE: when the matrix of the test sample is complex and strongly retained substances affect the subsequent detection, a gradient elution procedure can be adopted, see Appendix A.

5.5.2.3 Flow rate: 1.0 mL/min.

5.5.2.4 Column temperature: 35 °C.

5.5.2.5 Conditions of the evaporative light-scattering detector: set in accordance with the requirements of different brands of evaporative light-scattering detectors under high aqueous mobile phase conditions. For example, the atomization pressure is 0.137 MPa; the gain is 10; the evaporation temperature is 60 °C. Or those with equivalent performance.

5.5.2.6 Injection volume: 20 μL.

5.6 Drawing of Standard Curve

5.6.1 Differential detector

In accordance with the instrument reference conditions, determine the sucralose standard series of working solutions to obtain the chromatographic peak area of the corresponding standard series of working solutions. Take the mass concentration of the standard series of working solutions as the x-coordinate and the peak area response value as the y-coordinate to draw a working curve. For the chromatogram of sucralose standard solution, see Figure B.1 in Appendix B.

5.6.2 Evaporative light-scattering detector

In accordance with the instrument reference conditions, determine the sucralose standard series of working solutions to obtain the chromatographic peak area of the corresponding standard series of working solutions. Take the mass concentration of the standard series of working solutions as the x-coordinate and the peak area response value as the y-coordinate to draw a logarithmic working curve. For the chromatogram of sucralose standard solution, see Figure B.2.

g) of sucralose reference material, use water to dissolve it and transfer to a 25 mL volumetric flask. Reach a constant volume to the scale and evenly mix it. The stock solution is stored in a 4 °C refrigerator with a shelf life of 6 months.

10.4.2 Sucralose standard intermediate solution (10 mg/L): transfer-take 10.0 μL of sucralose standard stock solution (10.0 mg/mL) in a 10 mL volumetric flask, use water to reach a constant volume to the scale and evenly mix it. Store it in a 4 °C refrigerator with a shelf life of 3 months.

10.4.3 Sucralose standard series of working solutions: respectively transfer-take 0.100 mL, 0.200 mL, 0.500 mL, 1.00 mL and 1.50 mL of sucralose standard intermediate solution (10 mg/L) to 10 mL volumetric flasks, use water to reach a constant volume to the scale, and evenly mix them. Prepare sucralose standard series of working solutions with a mass concentration of 0.100 mg/L, 0.200 mg/L, 0.500 mg/L, 1.00 mg/L and 1.50 mg/L, respectively. Prepare them right before use.

10.5 Materials

10.5.1 Solid phase extraction column (column specification: 200 mg/6 mL, *N*-vinylpyrrolidone and divinylbenzene hydrophilic-lipophilic balanced packing or equivalent column).

10.5.2 0.22 μm hydrophilic microporous filter membrane and 0.22 μm hydrophobic microporous filter membrane.

11 Instruments and Equipment

11.1 High performance liquid chromatograph - tandem mass spectrometer: equipped with electrospray ion source.

11.2 Balance: with a division value of 0.1 mg and 1 mg, respectively.

11.3 Vortex mixer.

11.4 Water bath.

11.5 Ultrasonic generator: 50 kHz.

11.6 Centrifuge: with a speed ≥ 3,000 r/min.

11.7 Solid phase extraction device.

11.8 Homogenizer.

11.9 Pulverizer.

12 Analytical Procedures

12.1 Specimen Preparation

For liquid samples: shake them well; for semi-solid samples and powdery samples with uniform matrix: directly determine them; other samples need to be homogenized or evenly pulverized.

12.2 Specimen Extraction

12.2.1 Alcohol specimens

12.2.1.1 Distilled alcohol

Weigh-take 2 g (accurate to 0.001 g) of evenly mixed distilled alcohol specimen in a 10 mL volumetric flask, use water to dilute to the scale and shake it well. Filter the specimen through a 0.22 μm hydrophilic microporous filter membrane. The filtrate is the prepared specimen solution to be tested.

12.2.1.2 Fermented alcohol and integrated alcohol

12.2.1.2.1 For liquid specimens: weigh-take 2 g (accurate to 0.001 g) of evenly mixed specimen in an evaporating dish, heat it on a 60 °C water bath for 30 minutes, use 10 mL of water to rinse the residue in the evaporating dish in three times, and combine the washing fluids and transfer them to a 25 mL volumetric flask. Use water to reach a constant volume to the scale, shake it well and reserve it for purification.

12.2.1.2.2 For alcohol specimens containing solid substance: weigh-take 2 g (accurate to 0.001 g) of evenly mixed alcohol specimen in an evaporating dish, heat it on a 60 °C water bath for 30 minutes, use 10 mL of water to rinse the residue in the evaporating dish in three times, and combine the washing fluids and transfer them to a 15 mL centrifuge tube. On a vortex mixer, oscillate it for 3 minutes, perform ultrasonic extraction for 20 minutes, then, at 8,500 r/min, centrifuge for 5 minutes. Transfer the supernatant to a 25 mL volumetric flask, and use 10 mL of water to repeat the extraction once, combine the extracting solutions, then, use water to reach a constant volume to the scale, shake it well and reserve it for purification.

12.2.2 Jelly, candies and candied fruits specimens

Weigh-take 2 g (accurate to 0.001 g) of specimen in a 50 mL centrifuge tube and add 10 mL of water. On a vortex mixer, oscillate it for 3 minutes, then, add 1.00 mL of zinc acetate solution and 1.00 mL of potassium ferrocyanide solution. In a 60 °C water bath, heat it for 15 minutes. During the water bath, pay attention to shake and disperse it. Perform ultrasonic extraction for 20 minutes; at 8,500 r/min, centrifuge for 5 minutes. Take the supernatant (if necessary, use a fast quantitative filter paper to filter it) in a 25 mL volumetric flask, use 10 mL of water to repeat the extraction once. After combining the extracting solutions, add water to reach a constant volume to the scale. Evenly mix it, then transfer it to a 50 mL centrifuge tube. Add 15 mL of n-hexane, on a vortex mixer, oscillate it for 3 minutes. At 8,500 r/min, centrifuge for 5 minutes. Discard the upper n-hexane layer and reserve the subnatant for purification.

12.2.3 Other specimens

Weigh-take 2 g (accurate to 0.001 g) of specimen into a 50 mL centrifuge tube and add 10 mL

of water. On a vortex mixer, oscillate for 3 minutes, then add 1.00 mL of zinc acetate solution and 1.00 mL of potassium ferrocyanide solution. Perform ultrasonic extraction for 20 minutes; at 8,500 r/min, centrifuge for 5 minutes. Take the supernatant (if necessary, use a fast quantitative filter paper to filter it) in a 25 mL volumetric flask, use 10 mL of water to repeat the extraction once. After combining the extracting solutions, add water to reach a constant volume to the scale. Evenly mix it, then transfer it to a 50 mL centrifuge tube. Add 15 mL of n-hexane, on a vortex mixer, oscillate it for 3 minutes. At 8,500 r/min, centrifuge for 5 minutes. Discard the upper n-hexane layer and reserve the subnatant for purification.

12.3 Specimen Purification

Before use, the solid phase extraction column is successively activated with 4 mL of methanol and 4 mL of water to maintain the column moist.

Accurately transfer-take 10.00 mL of the specimen extracting solution into the activated solid phase extraction column and control the liquid flow rate to no more than 1 drop/s. After the liquid in the column is completely discharged, use 3 mL of methanol to elute it, collect the methanol eluent in a 10 mL volumetric flask, add water to reach a constant volume to the scale and evenly mix it. Use a 0.22 μm hydrophobic microporous filter membrane to filter it. The filtrate is the prepared specimen solution to be tested.

NOTE: the solution of jelly samples to be purified needs to be heated in a 50 °C water bath, then, pass through the column while it is still hot, otherwise, the extraction column will be easily blocked.

12.4 Blank Test

The pre-treatment of different specimens requires a blank test of the specimens to be simultaneously conducted.

12.5 Reference Conditions of Instruments

12.5.1 Liquid chromatography conditions

12.5.1.1 Chromatographic column: C_{18} (100 mm × 4.6 mm, 2.5 μm), or equivalent column.

12.5.1.2 Mobile phase: A is water, B is methanol. See the gradient elution procedure in Table 1.

12.5.1.3 Flow rate: 0.6 mL/min.

12.5.1.4 Column temperature: 40 °C.

12.5.1.5 Injection volume: 1 μL.

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