NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.298-2023

National food safety standard - Determination of sucralose in foods

Issued on: September 6, 2023 **Implemented on: September 6, 2024**

Issued by: NHC; SAMR

Table of Contents

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 C18 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.

3.2 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 (C12H19Cl3O8, CAS. 56038-13-2). purity ? 99%, or a standard substance certified by the state and awarded a reference material certificate.

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