National Standards of People's Republic of China

GB 22255-2014

National Food Safety Standard

Determination of Sucralose in Foods

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Issued by National Health and Family Planning Commission

Foreword

This standard will replace GB/T 22255-2008 Determination of Sucralose in Foods from the implementation date of this standard.

Compared with GB 2760—2007, the major changes in this standard are as follows:

- This standard changed the standard title into National Standard for Food Safety ----Determination of Sucralose in Foods

- This standard introduced the application of refractive index detectors.

- This standard added the solid-phase extraction column purification and isolation steps in test sample preparation.

- This standard added preparation steps for samples of fermented alcoholic drinks, integrated alcoholic drinks and protein free beverages.

National Standard for Food Safety

Determination of Sucralose in Foods

1. Scope

This standard specifies the method to determine sucralose in foods.

This standard applies to the determination of sucralose in foods.

2. Principle

By extracting sycralose from a food sample using methanol aqueous solution, removing protein and fat contained in the extract, purifying and enriching the extract using a solid-phase extraction column, separating the extract using a high performance liquid chromatograph and a C18 reverse-phase chromatographic column, then analyzing the extract using an evaporative light scattering detector or a refractive index detector, the qualitative and the quantitative analyses could be carried out based on the retention time and peak height/peak area respectively.

3. Reagents and materials

Unless otherwise specified, reagents used for the determination method specified herein are all analytical reagents, and water used is water for analytical laboratory use----Class 1as specified in GB/T6682.

3.1. Reagents

3.1.1 Methanol (CH_3OH)

3.1.2 Acetonitrile (CH_3CN):chromatographic grade

3.1.3 Hexane (C_6H_{14})

3.1.4 Zinc acetate: $Zn(CH_3COO)_2 \cdot 2H_2O$

3.1.5 Potassium Ferrocyanide:$K_4Fe(CN)_6 \cdot 3H_2O$

3.1.6 Neutral Alumina:100~200mesh

3.2. Reagent compounding

3.2.1 Zinc acetate solution (219g/L): mix 21.9g Zinc acetate and 3mL acetic acid and then dilute with water to 100mL.

3.2.2 Potassium ferrocyanide solution (106 g/L): Dilute 10.6g potassium ferrocyanide with water to 100mL.

3.2.3 Methanol-water solution (75+25): Mix up 25mL methanol and 75mL water.

3.2.4 Acetonitrile-water solution (11+89): Mix up 11mL acetonitrile and 89mL water.

3.3. Standard products

Standard Sucralose ($C_{12}H_{19}C_{13}O_8$): CAS No.: 56038-13-2, purity ≥99%

3.4. Formulation of standard solution

3.4.1 Sucralose Standard Stock Solution (10.0mg/mL): Transfer 0.25g (accurate to 0.0001g) standard sucralose to a 25mL volumetric flask, dilute with water to volume and mix up, the concentration will be 10.0mg/mL. Shelf life of the solution stored in a lab refrigerator at 4°C is 6 months.

3.4.2 Sucralose Inter-mediate Standard Solution: Transfer 5.00 mg/mL sucralose standard stock solution prepared in 3.4.1 to a 50mL volumetric flask, dilute with water to volume and mix up, the concentration will be 1.0mg/mL. Shelf life of the solution stored in a lab refrigerator at 4°C is 3 months.

3.4.3 Sucralose Standard Working Solutions: Transfer 0.200mL, 0.500mL, 1.00mL, 2.00mL and 4.00mL inter-mediate standard solution prepared in 3.4.2 to five 10mL volumetric flasks, respectively. Dilute each with water to volume and mix up, the concentration will be 0.0200mg/mL, 0.0500mg/mL, 0.100mg/mL, 0.200mg/mL,and 0.400mg/mL respectively.

3.5. Materials

Solid-phase extraction columns (200mg, using hydrophile-lipophile balanced N-Vinyl-2-pyrrolidone and divinylbenzene as packing) should be activated by 4mL methanol and 4mL water successively before use.

4. Equipment and facilities

4.1 High Performance Liquid Chromatograph:equipped with a refractive index detector/evaporative light scattering detector.

4.2 Balance Scale: a sensor volume is 0.1mg/1mg

4.3 Vortex Mixer

4.4 Centrifuge: rotating speed≥3,000r/min

4.5 Centrifuge: rotating speed≥10,000r/min

4.6 Ultra-sonic Cleaner: operating frequency:35KHz

4.7 Water Bath

5. Analytical procedures

5.1 Specimen preparation

5.1.1 Test Samples Containing Protein and Fat

5.1.1.1 Transfer a piece of 1~2g(accurate to 0.001g) solid sample crushed evenly and 1~5g liquid sample (accurate to 0.001g) to a 50mL centrifuge tube, add 5mL water, vortex on the vortex mixer for 3 min, then add 15mL methanol, and vortex on the vortex mixer again for 30 s; after an ultrasonic extraction for 20 min, centrifugate at 3000r/min for 10 min, then transfer the supernatant to a 50mL centrifuge tube. Add 5.0mL methanol-water solution(75+25) into the sediment, stir up with a glass rod, vortex on the vortex mixer for 30s, centrifugate at 3000r/min for 10 min, and do the extraction operation again, then collect and transfer all supernatants to a 150mL separatory funnel .

5.1.1.2 Add 30mL Hexane into the 150mL separatory funnel mentioned above, shake for 2 min, allow the mixture to stand for 20 minutes and separate into two layers, transfer the lower layer water phase into a 50mL evaporation dish; put the evaporation dish into the water bath for evaporation by boiling until only about 1mL liquid remained. Wash the evaporation dish with 9mL water totally in three flushing, collect and transfer the used water for the three flushing to a 15mL centrifuge tube. After an ultra-sonic treatment of 5min, centrifugate the used flushing water at 3000r/min for 10 min.

5.1.1.3 Inject all supernatants mentioned in 5.1.1.1 into an activated solid extraction column at a flow rate no more than 1 drop/second, add 1mL water when the supernatant level in the column reaches about 2mm, drain the extraction column keeping the flow rate at 1 drop/second. Then elute the extraction column with 3mL methanol, collected and transfer the used methanol eluent to a 50mL evaporation dish, put the evaporation dish into the water bath for evaporation by boiling until the used methanol eluent contained is dried up, dilute the evaporation residue with 1.0mL acetonitrile-water solution(11+89), and filter the solution with a 0.45μm membrance filter, the filtered solution is the prepared test sample.

Note: For jelly products, the supernatants extracted should be heated in the water bath at 50℃and pass through the extraction column when it is still hot to avoid plugging.

5.1.2 Sauce and Sauce Pickled Products, Soy Sauce & Vinegar

Transfer a 2g evenly mixed product sample (accurate to 0.001g) to a 50mL centrifuge tube, add 1.0g neutral alumina and 3mL water, vortex on the vortex mixer for 3min, then add 15mL methanol, and complete the procedure specified from 5.1.1.1 “and vortex on the vortex mixer again for 30 s; after an ultrasonic extraction for 20 min, centrifugate at 3000r/min for 10 min” to 5.1.1.3 “the filtered solution is the prepared test sample” in order.

5.1.3 Alcoholic Samples(fermented alcoholic drinks and integrated alcoholic drinks)

Transfer a 2g evenly mixed product sample (accurate to 0.001g) to a 50mL evaporation dish, put the evaporation dish into the water bath for evaporation by boiling until the sample is dried up, dilute the evaporation residue with 1.0mL acetonitrile-water solution(11+89), and filter the solution with a 0.45μm membrance filter, the filtered solution is the prepared test sample.

5.1.4 Non-alcoholic Beverages

Transfer a 2g evenly mixed product sample (accurate to 0.001g) to a 15mL centrifuge tube, add 5mL water, vortex on the vortex mixer for 30s, centrifugate at 3000r/min for 10 min, then complete steps specified in 5.1.1.3.

5.1.5 Flavored Fermented Milk and Milky Tea Products

Transfer a 1~5g evenly mixed product sample (accurate to 0.001g) to a 50mL centrifuge tube, add 5mL water, vortex on the vortex mixer for 3min, add 15mL methanol, 0.50 mL zinc acetate solution, and 0.50 mL potassium ferrocyanide solution, centrifugate at 3000r/min for 10 min, and complete the procedure specified from 5.1.1.1 “and vortex on the vortex mixer again for 30 s; after an ultrasonic extraction for 20 min, centrifugate at 3000r/min for 10 min” to 5.1.1.3 “the filtered solution is the prepared test sample” in order.

For various test samples, sample pre-treatment shall be done simultaneously with a blank test.

5.2 Reference conditions for equipment

5.2.1 Chromatographic Column: C18, (4.6×150mm, 5μm) or equivalent product

5.2.2 Mobile Phase: acetonitrile-water solution(89+11)

Note: In order to avoid influences of strong retention substances on subsequent testing steps, an elution procedure (applicable to evaporative light scattering detectors) could be to adopt after testing a sample with complex matrix.

5.2.3 Flow Rate: 1.0mL/min

5.2.4 Column Temperature: 35℃

5.2.5 Condition of Refractive Index Detector

----Temperature in Detection Cell：35℃

----Detection Sensitivity: 16

5.2.6 Condition of Evaporative Light Scattering Detector:

The detector should be set according to its brand-specific requirements for high water mobile phase.

For example, a Sedex 75 evaporative light scattering detector should be set as follows:

Atomization Pressure: 3.5Bar, Gain:8,Evaporation Temperature:80℃

Otherwise, a detector with equivalent performances should be used.

5.2.7 Injection Volume: 20.0LμL

5.3 Standard curve mapping

5.3.1 Refractive Index Detector

Inject 20.0μL of the 5 kinds of sucralose standard working solutions, respectively. Determine the peak area under the chromatographic conditions mentioned above, and establish the peak area-sucralose concentration (mg/mL) standard curve. As shown in Formula (1), the curve equation complies with working principles of the refractive index detector.

$$y = ax + b \qquad \cdots\cdots\cdots\cdots\cdots\cdots (1)$$

In the formula:

y-----refers to the peak area

a, b----constants related to detection cell temperature, mobile phase properties and other experimental conditions

x---sucralose concentration (mg/mL)

5.3.2 Evaporative Light Scattering Detector

Inject 20.0μL of the 5 kinds of sucralose standard working solutions, respectively. Determine the peak area under the given chromatographic conditions, and establish the peak area-sucralose concentration (mg/mL) standard curve. As shown in Formula (2), the curve equation complies with working principles of the evaporative light-scattering detector.

$$y = bx^{a} \qquad \cdots\cdots\cdots\cdots\cdots\cdots (2)$$

In the formula:

y-----refers to the peak area

a, b----constants related to evaporation chamber temperature, mobile phase properties and other experimental conditions

x---sucralose concentration (mg/mL)

Depending on the data processing method of software processing data collected by experimental instruments, the above mentioned Equation may be converted into a logarithmic equation, i.e. lg*y*=*b*+*a*lgx.

5.4 Testing of Sample Solutions

Inject 20.0μL sample solution and 20.0μL blank solution into a high performance liquid chromatograph , respectively and carry out high-performance liquid chromatographic evaluations. As specified in Annex B, the qualitative and the quantitative analyses shall be carried out based on the retention time and the peak area external reference method, respectively.

6. Statement of analysis result

The sucralose content in a test sample could be expressed by Formula (3)

$$X = \frac{(c - c_0) \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots\cdots\cdots\cdots\cdots (3)$$

In the formula:

X----refers to the sucralose content in a test sample

c----refers to the sucralose concentration positioned on the standard curve, in the liquid injected as sample, (mg/mL)

c_0----refers to the sucralose concentration positioned on the standard curve, in the liquid injected as blank sample, (mg/mL)

V----refers to the test sample's constant volume, (mL)

m----mass of the test sample, (g)

1000----conversion coefficient

The calculation results should be reserved 3-digit decimals.

7. Precision

The absolute difference between two independent determination results obtained based on the repeatability of the determination procedure should not exceed 10% of these tow results' mathematic average.

8. Others

If dilute a sample of 2g with water to 1.00 mL, the detection limit is 0.0025 g/kg, and the quantitation limit is 0.0075 g/kg.

Appendix A

Elution Procedure of the Liquid Chromatograph under Complex Conditions (applicable to evaporative light scattering detectors)

In order to avoid influences of strong retention substances on subsequent testing steps, an elution procedure could be to adopt after testing a sample with complex matrix. Elution parameters are as shown in Table A.1.

Table A.1 Elution Procedure of the Liquid Chromatograph under Complex Conditions (applicable to evaporative light scattering detectors)

Time (min)	Ultra-pure Water (Volume Fraction) %	Acetonitrile (Volume Fraction) %
0	89	11
14	89	11
15	10	90
22	10	90
23	89	11
26	89	11

Appendix B

High Performance Liquid Chromatogram of Sucralose

Refer to Fig B.1 for liquid chromatogram (evaporative light scattering detector) of standard sucralose solution (0.200mL).

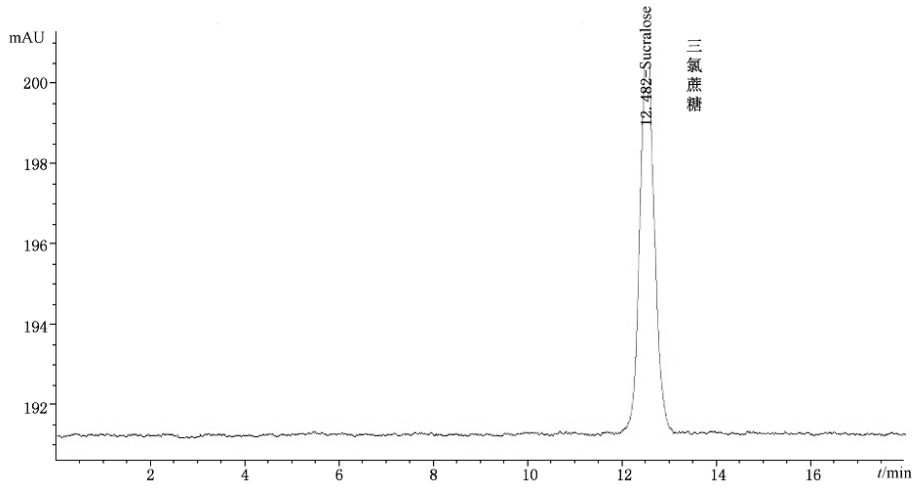

Fig B.1 Liquid Chromatogram(evaporative light scattering detector) of Standard Sucralose Solution

Refer to Fig B.2 for liquid chromatogram (refractive index detector) of standard sucralose solution (0.200mL).

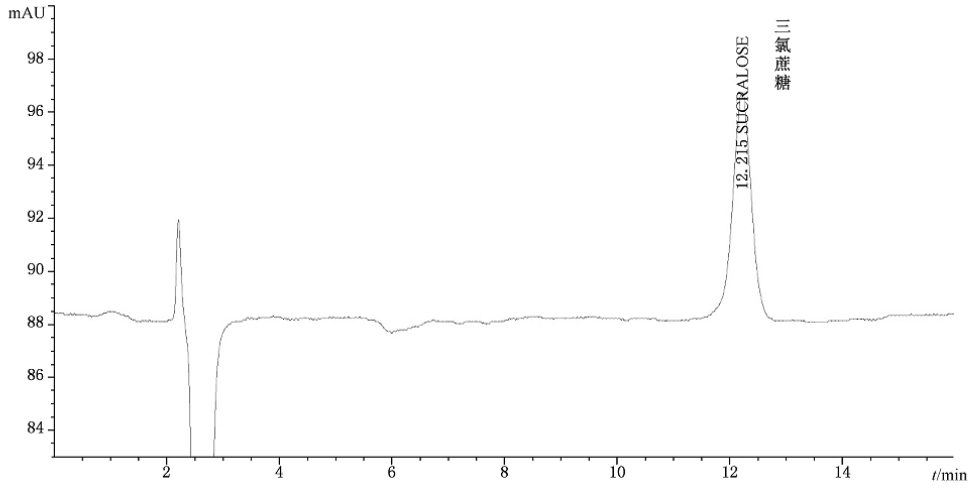

Fig B.2 Liquid Chromatogram (refractive index detector) of Standard Sucralose Solution