GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.28-2016

National Food Safety Standard - Determination of Benzoic Acid, Sorbic Acid and Saccharin Sodium in Foods

Issued on: December 23, 2016 **Implemented on: June 23, 2017**

Issued by: National Health and Family Planning Commission of the People's Republic of China;

China Food and Drug Administration.

Table of Contents

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1 Scope

This Standard specifies the determination method for benzoic acid, sorbic acid AND sodium saccharin in foods.

The first method of this Standard is applicable to the determination of benzoic acid, sorbic acid AND saccharin sodium in foods; the second method is applicable to the determination of benzoic acid and sorbic acid in soy sauce, fruit juice AND jam.

First method – Liquid chromatography

2 Principle

Use water to extract the sample; use n-hexane to degrease high-fat sample; use protein precipitation agent to precipitate the protein of high-protein sample; adopt liquid chromatography to perform separation; use UV detector to perform detection; use external standard method to determine the quantity.

3 Reagents and Materials

Unless otherwise stated, the used reagents in this method shall all be analytical purity; the water shall be the Grade-1 water which is specified in GB/T 6682.

3.1 Reagents

3.1.1 Ammonia ($NH_3 \cdot H_2O$).

3.1.2 Potassium ferrocyanide [$K_4Fe(CN)_6 \cdot 3H_2O$].

3.1.3 Zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$].

3.1.4 Anhydrous ethanol (CH_3CH_2OH).

3.1.5 N-hexane (C_6H_{14}).

3.1.6 Methanol (CH_3OH): chromatographic purity.

5 Analysis Steps

5.1 Preparation of sample

Take a plurality of prepackaged homogeneous samples, such as beverages and liquid milk, etc.; mix them directly; use tissue homogenizer to homogenize the non-homogeneous-liquid and semi-solid samples; use grinder to fully comminute and stir the solid sample evenly; perform heating and melting - 50°C ~ 60°C - for cheese, butter AND chocolate, etc.; when it is hot, stir it evenly. Take 200g of it into a glass container and seal it. The liquid sample shall be stored at 4°C; the rest sample shall be stored at -18°C.

5.2 Sample's extraction

5.2.1 General Samples

Accurately weigh and take about 2g (accurate to 0.001g) of sample in a 50mL stoppered centrifuge tube; add about 25mL of water; vortex-mix it; in 50°C water bath, sonicate it for 20min; cool it to room temperature; then, add 2mL of potassium ferrocyanide solution AND 2mL of zinc acetate solution; mix them; centrifuge it at 8000r/min for 5min; transfer the aqueous phase into a 50mL volumetric flask; add 20mL of water in the residue; vortex-mix it; then sonicate it for 5min; centrifuge it at 8000r/min for 5min; transfer the aqueous phase into the same 50mL volumetric flask; use water to dilute it to the scale; mix it evenly. Take appropriate amount of supernatant to pass the 0.22μm filter; wait for the determination of liquid chromatography.

Note: When carbonated drinks, fruit wines, fruit juices and distilled spirits, etc. are determined, protein precipitants may not be added.

5.2.2 Samples of gum-base jelly and candy, etc.

Accurately weigh and take about 2g (accurate to 0.001g) of sample in a 50mL stoppered centrifuge tube; add about 25mL of water; vortex-mix it; in 70°C water bath, heat and dissolve the sample; in 50°C water bath, sonicate it for 20min. Subsequent operations shall be the same as those in 5.2.1.

5.2.3 High-fat sample such as fat, chocolate, butter and fried food, etc.

Accurately weigh and take about 2g (accurate to 0.001g) of sample in a 50mL stoppered centrifuge tube; add 10mL of n-hexane; in 60°C water bath, heat it for 5min; gently and occasionally shake it to dissolve fat; then add 25mL of ammonia solution (1+99) AND 1mL of ethanol; vortex-mix them; in 50°C water bath, sonicate it for 20min; cool it to room temperature; add 2mL of potassium ferrocyanide solution AND 2mL of zinc acetate solution; mix them evenly; at 8000r/min 5min, centrifuge it for 5min; discard the organic phase; transfer the aqueous phase into a 50 mL volumetric flask;

10.1.2 Ethanol (C_2H_5OH).

10.1.3 N-hexane (C_6H_{14}).

10.1.4 Ethyl acetate ($CH_3CO_2C_2H_5$): chromatographic purity.

10.1.5 Hydrochloric acid (HCl).

10.1.6 Sodium chloride (NaCl).

10.1.7 Anhydrous sodium sulfate (Na_2SO_4): at 500°C, dry for 8 hours; in desiccator, after it is cooled to room temperature, it shall be set for standby application.

10.2 Preparation of reagent

10.2.1 Hydrochloric acid solution (1+1): take 50mL of hydrochloric acid; at the time of stirring it, slowly add it into 50mL water; mix it evenly.

10.2.2 Sodium chloride solution (40g/L): weigh 40g of sodium chloride; use proper amount of water to dissolve with; add 2mL of hydrochloric acid solution; add water to set the constant volume to 1L.

10.2.3 N-hexane-ethyl acetate mixed solution (1+1): take 100 mL of n-hexane AND 100 mL of ethyl acetate; mix them evenly.

10.3 Standard products

10.3.1 Benzoic acid (C_6H_5COOH, CAS No.: 65-85-0): the purity shall be ≥ 99.0%; OR the standard substance which is certified by the country AND granted for standard substance certificate.

10.3.2 Sorbic acid ($C_6H_8O_2$, CAS No.: 110-44-1): the purity shall be ≥ 99.0%; OR the standard substance which is certified by the country AND granted for standard substance certificate.

10.4 Preparation of standard solution

10.4.1 Benzoic acid AND sorbic acid standard stock solution (1000mg/L): respectively and accurately weigh and take 0.1g of benzoic acid AND sorbic acid (accurate to 0.0001g); use methanol to dissolve them AND dilute them to 100 mL respectively; Transfer them into a sealed container; at -18°C, store it. The preservation period shall be 6 months.

10.4.2 Mixed standard intermediate-solution (200mg/L) of benzoic acid AND sorbic acid: respectively and accurately pipette 10.0mL of benzoic acid and sorbic acid standard stock solution into a 50mL volumetric flask; use ethyl acetate to dilute to constant volume. Transfer it into a sealed container; at -18°C, store it. The preservation

anhydrous sodium sulfate. Add ethyl ether to wash the anhydrous-sodium-sulfate layer; collect it to the scale of about 25mL; finally, use diethyl ether to set the constant volume; mix it evenly. Accurately pipette 5mL of ethyl ether in a 5mL stoppered test tube; at 35°C, use nitrogen to blow it until dryness; add 2mL of n-hexane-ethyl acetate (1+1) mixed solution to dissolve the residue; wait for the determination of gas chromatography.

12.3 Reference conditions of instruments

12.3.1 Chromatographic column: polyethylene-glycol capillary gas chromatography column; inner diameter - 320μm; length - 30 m; membrane's thickness - 0.25μm; OR the chromatographic column with equivalent performance.

12.3.2 Carrier gas: nitrogen; flow rate - 3mL/min.

12.3.3 Air: 400L/min.

12.3.4 Hydrogen: 40L/min.

12.3.5 Inlet temperature: 250°C.

12.3.6 Detector temperature: 250°C.

12.3.7 Column temperature program: the initial temperature is 80 °C; hold for 2min; at a rate of 15°C/min, it shall be heated to 250°C; hold for 5min.

12.3.8 Sample volume: 2μL.

12.3.9 Split ratio: 10:1.

12.4 Preparation for standard curves

Respectively inject the mixed-standard-series working solution into the liquid chromatograph; treat the mass concentration as abscissa AND treat the peak area as ordinate; draw the standard curve.

12.5 Determination of sample solution

Inject the sample solution into the liquid chromatograph; obtain the peak area; according to the standard curve, obtain the mass concentration of benzoic acid and sorbic acid in test solution.

13 Expression of Analysis Results

Content of benzoic acid and sorbic acid in sample shall be calculated according to