GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.252-2016

National Food Safety Standard - Determination of Levulinic Acid in Foods

食品安全国家标准

食品中乙酰丙酸的测定

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Foreword

This Standard replaces SB/T 10417-2007, Method for Determination of β-acetylpropionic Acid in Soy Sauce.

Compared with SB/T 10417-2007, the major changes of this Standard are as follows:

-- Change the standard name into “National Food Safety Standard - Determination of Levulinic Acid in Foods”;

-- Extend the scope to soy sauce, beverage (including carbonate beverage, coffee beverage, juice, milk beverage, vegetable protein beverage), sauce, honey, milk powder, biscuit, pastry, candy, jelly and flour product;

-- Add reagents’ grade and molecular formula;

-- Add preparation method of liquid sample and solid sample;

-- Add quantitation-limit of the determination method of levulinic acid in foods;

-- Modify the chromatogram.

National Food Safety Standard - Determination of Levulinic Acid in Foods

1 Application Scope

This Standard specifies the method to use gas chromatography for the test of levulinic acid in foods.

This Standard applies to the test of levulinic acid in soy sauce, beverage (including carbonate beverage, coffee beverage, juice, milk beverage, vegetable protein beverage), sauce, honey, milk powder, biscuit, pastry, candy, jelly and flour product.

Method I -- Internal Standard Method

2 Principle

After the sample is acidized, use ether to extract levulinic acid; use heptanoic acid as the internal standard substance; use gas chromatography equipped with hydrogen flame ionization detector to test; use internal standard method to quantify.

3 Reagents and Materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-2 water specified by GB/T 6682.

3.1 Reagents

3.1.1 Anhydrous sodium sulfate (Na_2SO_4): burn for 4 h at 650°C; store in the dryer as a standby.

3.1.2 Anhydrous ether ($C_4H_{10}O$).

3.1.3 Hydrochloric acid (HCl).

3.1.4 Sodium chloride (NaCl)

3.1.5 Ethyl acetate ($C_4H_8O_2$): chromatographically pure.

3.2 Preparation of reagents

sodium chloride to wash for twice; discard the lower layer; for ether layer, use 30 g of anhydrous sodium sulfate to dehydrate; concentrate to near-dryness at about 45°C; use ethyl acetate to fix-volume to 10 mL; shake to mix well; use gas chromatograph to test the sample.

5.1.2 Solid sample

Accurately weigh 5 g (accurate to 0.001 g) of fully pulverized sample into a 100 mL test tube with plug; add 10 mL of saturated sodium chloride solution, 1.0 mL of heptanoic acid standard stock solution, 3.0 mL of hydrochloric acid; shake-mix well for 1 min; add 50 mL of ether; shake the mixture for 3 min ~ 5 min; let stand for about 10 min ~ 15 min; after layering, extract the upper-layer ether extract into a 250 mL separatory funnel; then, repeat the extraction twice, each time use 50 mL of ether; combine the ether extract; use 10 mL of saturated sodium chloride solution to wash twice; discard the lower layer; use 30 g of anhydrous sodium sulfate to dehydrate; concentrate to about dryness at about 45°C; for ether layer, use ethyl acetate to fix-volume to 10 mL; shake-mix well; use gas chromatograph to test the sample.

5.2 Gas chromatograph reference conditions

5.2.1 Chromatographic column: DB-WAX quartz capillary column (30 m × 0. 25 mm × 0. 25 μm) or chromatographic column of similar model.

5.2.2 Temperature of the sample injector: 270°C.

5.2.3 Temperature of the detector: 270°C.

5.2.4 Temperature-rise procedure: initial column-temperature is 120°C, maintain for 1 min; rise the temperature to 230°C at 8°C/min, maintain for 4 min.

5.2.5 Carrier gas: high-purity nitrogen; purity ≥ 99.999%; flow velocity: 1.0 mL/min.

5.2.6 Hydrogen flow velocity: 30 mL/min.

5.2.7 Air flow velocity: 300 mL/min.

5.2.8 Make-up gas flow velocity: 30 mL/min.

5.2.9 Injection method: split injection; split ratio: 25:1.

5.2.10 Injection volume: 1 μL.

5.3 Preparation of standard curve

Inject standard series working solutions into gas chromatograph separately; test the corresponding peak areas of levulinic acid and internal standard substance; use levulinic acid peak area / internal standard substance peak area as the vertical ordinate, levulinic acid mass concentration as the abscissa, draw the standard curve.

Method II -- External Standard Method

9 Principle

After the sample is acidized, use ether to extract levulinic acid; use gas chromatograph, equipped with hydrogen flame ionization detector, to conduct separation determination; use external standard method to quantify.

10 Reagents and Materials

Unless otherwise specified, all the reagents in this method are analytical reagents; the water used is grade-2 water specified by GB/T 6682.

10.1 Reagents

10.1.1 Anhydrous ether ($C_4H_{10}O$).

10.1.2 Anhydrous sodium sulfate (Na_2SO_4): burn for 4h at 650°C; store in the dryer as standby.

10.1.3 Ethyl acetate ($C_4H_8O_2$): chromatographically pure.

10.1.4 Hydrochloric acid (HCl).

10.1.5 Sodium chloride (NaCl).

10.2 Preparation of reagents

Hydrochloric acid solution (6 mol/L): measure 50 mL of hydrochloric acid; use water to dilute to 100 mL.

10.3 Standard

Levulinic acid ($C_5H_8O_3$): purity ≥ 99.5%.

10.4 Preparation of standard solution

10.4.1 Levulinic acid standard solution (5.00 mg/mL): accurately weigh 0.5 g of levulinic acid (accurate to 0.000 1 g); use ethyl acetate to dissolve; fix-volume to a 100 mL volumetric flask; mix up well.

10.4.2 Levulinic acid standard series working solutions:

Respectively and accurately absorb 0.0 mL, 0.05 mL, 0.1 mL, 0.5 mL, 1.0 mL and 1.5 mL of standard stock solutions of levulinic acid (10.4.1) into 6 10 mL volumetric flasks;

× 0.25 μm) or chromatographic column of similar models.

12.2.2 Temperature of the sample injector: 260°C.

12.2.3 Temperature of the detector: 280°C.

12.2.4 Temperature-rise procedure: initial column temperature is 60°C, maintain for 1 min; rise the temperature to 220°C at 18°C /min; maintain for 1 min; rise the temperature to 230°C at 10°C /min; maintain for 12 min.

12.2.4 Carrier gas: high purity nitrogen, purity ≥ 99.999%; flow velocity: 1.0 mL/min.

12.2.6 Hydrogen flow velocity: 30 mL/min.

12.2.7 Air flow velocity: 300 mL/min.

12.2.8 Make-up gas flow velocity: 30 mL/min.

12.2.9 Injection method: split injection; split ratio: 25:1.

12.2.10 Injection volume: 1 μL.

12.3 Preparation of standard curve

Inject standard series working solution into gas chromatograph separately; test the corresponding peak areas; use the mass concentration of standard working solution as the vertical ordinate, the corresponding peak area as the abscissa; draw the standard curve.

See Figure A.2 for the gas chromatogram of levulinic acid standard solution.

12.4 Test of sample solution

Inject the sample solution into the gas chromatograph; obtain the corresponding peak areas; get the mass concentration of levulinic acid in the to-be-test solution according to the standard curve.

13 Description of the analysis result

Calculate content of levulinic acid according to Equation (2):

$$X = \frac{\rho \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots(2)$$

Where:

X -- the content of levulinic acid in the sample; the unit is mg/kg;

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