GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.121-2016

Food safety national standard –

Determination of dehydroacetic acid in foods

Issued on: August 31, 2016 **Implemented on: March 1, 2017**

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

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Foreword

This Standard replaces GB/T 5009.121-2003 "Determination of dehydroacetic acid in foods".

Compared with GB/T 5009.121-2003, the main changes in this Standard are as follows:

- modified the standard name as "Food safety national standard - Determination of dehydroacetic acid in foods";
- added liquid chromatography;
- the liquid chromatography used the method of GB/T 23377-2009;
- added specimen classification;
- improved the preparation and extraction method of sample in gas chromatography;
- modified the chromatographic conditions of gas chromatography.

Food safety national standard –

Determination of dehydroacetic acid in foods

1 Scope

This Standard specifies the determination method of dehydroacetic acid in fruit and vegetable juice, fruit and vegetable pulp, pickle, fermented soy product, butter, bread, cake, baked food stuffing, compound seasoning, pre-made meat product, and cooked meat product.

This Standard is applicable to the determination of dehydroacetic acid in fruit and vegetable juice, fruit and vegetable pulp, pickle, fermented soy product, butter, bread, cake, baked food stuffing, compound seasoning, pre-made meat product, and cooked meat product. Other foods shall refer for implementation.

Method One -- Gas chromatography

2 Principle

Solid (semi-solid) sample, after protein settlement and defatting, is extracted with ethyl acetate. After acidified, the samples of fruit and vegetable juice and fruit and vegetable pulp are extracted with ethyl acetate. Use a gas chromatograph with hydrogen flame ionization detector to separate and determine. Determine the nature with the retention time of chromatographic peak. Quantify by external standard method.

3 Reagents and materials

Unless otherwise indicated, the reagents used in this method are all analytically pure, and water is Grade Two water specified in GB/T 6682.

3.1 Reagents

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

3.1.2 Hexane (C_6H_{14}): chromatographically pure.

3.1.3 Hydrochloric acid (HCl).

3.1.4 Zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

5 Analysis steps

5.1 Specimen preparation

5.1.1 Fruit and vegetable juice, fruit and vegetable pulp: weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 50mL centrifuge tube. Add 10 mL of water and shake 1 min. After adding 1 mL of hydrochloric acid solution (3.2.1) for acidification, accurately add 5.0 mL of ethyl acetate. Shake and extract 2 min. Place still and perform stratification. Take supernatant for the determination of gas chromatography.

5.1.2 Pickle, fermented soy product: use stainless steel homogenizer for sample homogenization. Weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 50mL centrifuge tube. Add about 15 mL of water, 2.5 mL of zinc sulfate solution (3.2.2). Adjust pH to 7.5 with sodium hydroxide solution (3.2.3). Perform ultrasonic extraction 15 min. Transfer to a 25mL volumetric flask. Add water to set volume. Transfer the sample solution into a centrifuge tube and centrifuge at 4000 r/min for 10 min. Take 10 mL of supernatant. After adding 1 mL of hydrochloric acid solution (3.2.1) for acidification, accurately add 5.0 mL of ethyl acetate. Shake 2 min. Place still and perform stratification. Take supernatant for the determination of gas chromatography.

5.1.3 Bread, pastries, baked goods filling, compound seasoning, pre-made meat product and cooked meat product: use a grinder to grind the sample or homogenize the sample with a stainless steel high-speed homogenizer. Weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 50mL centrifuge tube. Add into 15 mL of water, 2.5 mL of zinc sulfate solution (3.2.2). Adjust pH to 7.5 with sodium hydroxide solution (3.2.3). Perform ultrasonic extraction 15 min. Transfer to a 25mL volumetric flask. Add water to set volume. Transfer the sample into a separatory funnel. Add 5 mL of n-hexane. Shake 1 min. Place still and perform stratification. Take the lower aqueous phase and place it in a centrifuge tube. Centrifuge at 4000 r/min for 10 min. Take 10 mL of supernatant. After adding 1 mL of hydrochloric acid solution (3.2.1) for acidification, accurately add 5.0 mL of ethyl acetate. Shake 2 min. Place still and perform stratification. Take supernatant for the determination of gas chromatography.

5.1.4 Butter: weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 50mL centrifuge tube. Add into 15 mL of water, 2.5 mL of zinc sulfate solution (3.2.2). Adjust pH to 7.5 with sodium hydroxide solution (3.2.3). Perform ultrasonic extraction 15 min. Transfer to a 25mL volumetric flask. Add water to set volume. Transfer the sample into a separatory funnel. Add 5 mL of n-hexane. Shake 1 min. Place still and perform stratification. Take the lower aqueous phase and place it in a centrifuge tube. Centrifuge at 4000 r/min for 10 min. Take 10 mL of supernatant. After adding 1 mL of hydrochloric acid solution (3.2.1) for acidification, accurately add 5.0 mL of ethyl acetate. Shake 2 min.

9 Principle

The dehydroacetic acid in the sample is extracted with sodium hydroxide solution, delipidated, deproteinized, and membraned. Determine it with high performance liquid chromatography that is with UV or diode array detector. Determine the nature with the retention time of chromatographic peak. Quantify by external standard method.

10 Reagents and materials

Unless otherwise indicated, the reagents used in this method are all analytically pure, and water is Grade One water specified in GB/T 6682.

10.1 Reagents

10.1.1 Methanol (CH_4O): chromatographically pure.

10.1.2 Ammonium acetate ($C_2H_6O_2N$): superior-grade pure.

10.1.3 Sodium hydroxide (NaOH).

10.1.4 Hexane (C_6H_{14}).

10.1.5 Formic acid (CH_2O_2).

10.1.6 Zinc sulfate ($ZnSO_4 \cdot 7H_2O$).

10.2 Reagent preparation

10.2.1 Ammonium acetate solution (0.02 mol/L): weigh 1.54 g of ammonium acetate, dissolve in water and dilute to 1 L.

10.2.2 Sodium hydroxide solution (20 g/L): weigh 20 g of sodium hydroxide, dissolve in water and dilute to 1 L.

10.2.3 Formic acid solution (10%): measure 10 mL of formic acid, add 90 mL of water, mix well.

10.2.4 Zinc sulfate solution (120 g/L): weigh 120 g of zinc sulfate, dissolve in water and dilute to 1 L.

10.2.5 Methanol solution (70%): measure 70 mL of methanol, add 30 mL of water, mix well.

10.3 Standard product

Dehydroacetic acid ($C_8H_8O_4$, CAS: 520-45-6) standard product: purity ≥99.5%.

with 5 mL of water and elute with 2 mL of 70% methanol solution (10.2.5). Collect 2 mL of eluent. Perform vortex mixing. Filter with 0.45μm organic membrane for the determination of high performance liquid chromatography.

12.1.2 Pickle, fermented soy product: use stainless steel homogenizer for sample homogenization. Weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 25mL centrifuge tube. Add about 10 mL of water, 5 mL of zinc sulfate solution (10.2.4). Adjust pH to 7.5 with sodium hydroxide solution (10.2.2). Transfer to a 25mL volumetric flask. Add water to dilute to the scale. Shake well. Place in a 25mL centrifuge tube. Perform ultrasonic extraction for 10 min. Centrifuge at 4000 r/min for 10 min. Take supernatant and make it through 0.45μm organic membrane for the determination of high performance liquid chromatography.

12.1.3 Bread, pastries, baked goods fillings, compound seasonings: use a grinder to grind the sample or homogenize the sample with a stainless steel high-speed homogenizer. Weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 25mL centrifuge tube (if it needs filtering through solid phase extraction column, then use a 50mL centrifuge tube). Add about 10 mL of water, 5 mL of zinc sulfate solution (10.2.4). Adjust pH to 7.5 with sodium hydroxide solution (10.2.2). Transfer to a 25mL volumetric flask (if it needs filtering through solid phase extraction column, then use a 50mL volumetric flask). Add water to dilute to the scale. Shake well. Place it in a centrifuge tube. Perform ultrasonic extraction for 10 min. Transfer to the separatory funnel. Add 10 mL of n-hexane. Shake for 1min. Place still and perform stratification. Discard the n-hexane layer. Add 10 mL of n-hexane to repeat once. Take the lower aqueous phase in the centrifuge tube. Centrifuge at 4000 r/min for 10 min. Take supernatant and filter it through 0.45μm organic membrane for the determination of high performance liquid chromatography. If the separation of high performance liquid chromatographic is not ideal, take 20 mL of supernatant. Adjust pH to 5 with 10% formic acid (10.2.3). Set volume to 25 mL. Take 5mL of activated solid phase extraction column. Rinse with 5 mL of water and elute with 2 mL of 70% methanol solution (10.2.5). Collect 2 mL of eluent. Perform vortex mixing. Filter with 0.45μm organic membrane for the determination of high performance liquid chromatography.

12.1.4 Butter: weigh 2g ~ 5g of sample (to the nearest of 0.001 g) and place in a 25mL centrifuge tube (if it needs filtering through solid phase extraction column, then use a 50mL centrifuge tube). Add into about 10 mL of water, 5 mL of zinc sulfate solution (10.2.4). Adjust pH to 7.5 with sodium hydroxide solution (10.2.2). Transfer to a 25mL volumetric flask (if it needs filtering through solid phase extraction column, then use a 50mL volumetric flask). Add water to dilute to the scale. Shake well. Place it in a centrifuge tube. Perform ultrasonic extraction for 10 min. Transfer to the separatory funnel. Add 10 mL of n-hexane. Shake for 1min. Place still and perform stratification. Discard the n-hexane layer. Add 10 mL of n-hexane to repeat once. Take the lower aqueous phase in the

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