GB

NATIONAL STANDARD OF THE

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National Food Safety Standard - Food Contact Materials and Products - Determination of Migration of Nine Antioxidants

食品安全国家标准 食品接触材料及制品 9 种抗氧化剂迁移量的测定

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National Food Safety Standard - Food Contact Materials and Products - Determination of Migration of Nine Antioxidants

1 Scope

This Standard specifies methods for the determination of migration of nine antioxidants in food contact materials and products.

This Standard applies to the determination of migration of antioxidant 2246, antioxidant 264, butylated hydroxyanisole (BHA), 2,4-di-tert-butylphenol, antioxidant 425, antioxidant 300, antioxidant 1010, antioxidant 1076, and antioxidant 168.

Method-I High Performance Liquid Chromatography

2 Principle

After food contact materials and products undergo migration tests in accordance with GB 31604.1 and GB 5009.156, high performance liquid chromatography is used for detection. Among them, after filtration of water, 4% (volume fraction) acetic acid, 10% (volume fraction) ethanol, 20% (volume fraction) ethanol, 50% (volume fraction) ethanol food simulant and chemical alternative solvent 95% (volume fraction) ethanol soaking solution, the sample is injected directly. After the nitrogen blowing of the chemical alternative solvent isooctane soaking solution, dissolve it in methanol; and then inject the sample. Detect by UV detector or diode array detector, and quantified by peak area external standard method.

3 Reagents and Materials

Unless otherwise stated, all reagents are chromatographically pure. The water is Class-I water specified in GB/T 6682. During the test, plastic materials shall be avoided as much as possible for containers and transfer equipment.

3.1 Reagents

3.1.1 Foods containing ethanol, acidic food simulants and chemical alternative solvents: the reagents used must comply with the provisions of GB 5009.156.

3.1.2 Methanol (CH_4O).

3.1.3 Ethyl acetate ($C_4H_8O_2$).

3.2 Preparation of reagent

Foods containing ethanol, acidic food simulants and chemical alternative solvents: Prepared according to the requirements of GB 5009.156.

3.3 Standard product

9 antioxidant standard products (see Table A.1 in Appendix A), with purity ≥98%, or standard products certified by the country and awarded a reference material certificate.

3.4 Preparation of standard solution

3.4.1 9 Antioxidant standard stock solutions (1000 mg/L): Accurately weigh 25mg of each of the 9 antioxidant standard products (accurate to 0.1mg); dissolve them in 1mL of ethyl acetate. And then transfer to 9 pieces of 25mL brown volumetric flask; and make constant volume by adding methanol and shake well. Transfer the solution to a brown glass container and store it in an airtight container away from light in a 4°C refrigerator. The shelf life is 6 months.

3.4.2 9 antioxidant standard intermediate solution A (solvent is methanol): Accurately pipet 2.50mL of each of BHA and antioxidant 300 standard stock solutions, and 5.00mL of each of the other 7 antioxidants standard stock solutions into the same 50mL brown volumetric flask; make constant volume by methanol to obtain standard intermediate solution A of BHA and antioxidant 300 with mass concentration of 50.0 mg/L, and of the other 7 antioxidants with the mass concentration of 100 mg/L. Transfer the solution to a brown glass container and store it in an airtight container away from light in a 4°C refrigerator. The storage period is 3 months.

3.4.3 9 antioxidant standard intermediate solution B (solvent is ethyl acetate): Accurately pipet 2.50mL of each of BHA and antioxidant 300 standard stock solutions, and 5.00mL of each of the other 7 standard stock solutions into the same 50mL brown volumetric flask; make constant volume by ethyl acetate to obtain a standard intermediate solution B of BHA and antioxidant 300 with mass concentration of 50 mg/L, and the other 7 antioxidants with mass concentration of 100 mg/L. Transfer the solution to a brown glass container and store it in an airtight container away from light in a 4°C refrigerator. The storage period is 3 months.

3.4.4 Preparation of standard working solution

3.4.4.1 Chemical alternative solvent isooctane standard working solution

Accurately transfer 0.03mL, 0.1mL, 0.3mL, 1mL, and 3mL of 9 antioxidant standard intermediate solutions B into 5 pieces of 10mL volumetric flasks; and make constant volume with isooctane to obtain the standard working solutions of BHA and antioxidant 300 with mass concentration of 0.15 mg/L, 0.50 mg/L, 1.5mg/L, 5mg/L, 15mg/L, respectively; and other 7

antioxidants with the mass concentration of 0.30mg/L, 1.0mg/L, 3.0mg/L, 10mg/ L, 30mg/L, respectively. Accurately transfer 2.00mL of each standard working solution into 5 pieces of 10mL test tubes; blow with nitrogen at 40°C until nearly dry; then add 2.00mL of methanol to dissolve. Vortex and shake to reconstitute; and filter through a needle nylon filter; and then waiting to be measured on the machine.

3.4.4.2 Standard working solution of other food simulants and chemical alternative solvents 95% (volume fraction) ethanol

Accurately transfer 0.03mL, 0.1mL, 0.3mL, 1mL, and 3mL of 9 antioxidant standard intermediate solutions A into 5 pieces of 10mL volumetric flasks; and make constant volume with 10% ethanol; and obtain the standard working solution of BHA and antioxidant 300 with mass concentrations of 0.15mg/L, 0.50mg/L, 1.5 mg/L, 5 mg/L, 15 mg/L, respectively; and other 7 antioxidants with mass concentrations of 0.30 mg/L, 1.0 mg/L, and 3.0mg/L , 10mg/L, 30mg/L, respectively. Using the same method, prepare 9 antioxidant standard working solutions of the same concentration series by water, 4% acetic acid, 10% ethanol, 20% ethanol, 50% ethanol food simulant and chemical alternative solvent 95% ethanol. Take 1 mL of each standard working solution; filter it through a needle nylon filter; and then wait for measurement on the machine.

4 Instruments and Equipment

4.1 High performance liquid chromatograph: Equipped with UV detector or diode array detector.

4.2 Analytical balance: Sensitivity is 0.0001g.

4.3 Nitrogen blowing concentrator.

4.4 Glass micro-syringe: 10μL, 100μL and 1000μL.

4.5 Thermostatic equipment.

4.6 Vortex oscillator.

4.7 Needle nylon filter: Pore size is 0.22μm.

5 Analytical Procedure

5.1 Preparation of test solution

5.1.1 Migration test

Food contact materials and products undergo migration tests in accordance with the requirements of GB 31604.1 and GB 5009.156. If the soaking solution obtained from the

d) Column temperature: 40℃;

e) Injection volume: 50μL.

f) Detection wavelength: 287nm.

5.3 Drawing of standard curve

According to the instrument reference conditions listed in 5.2, inject the standard series working solutions into the high-performance liquid chromatograph respectively; measure the corresponding peak areas; and draw the standard working curve with the antioxidant concentration as the abscissa and the corresponding chromatographic peak area as the ordinate; and obtain the linear regression equation.

5.4 Determination of specimen solution

5.4.1 Qualitative determination

According to the instrument reference conditions listed in 5.2, measure the food simulant specimen solution and standard working solution, respectively. If the chromatographic peak retention time deviation between the food simulant sample solution and the standard solution with equivalent concentration is within the range of ±2.5%, the sample can be judged to contain corresponding objects to be measured. The HPLC chromatograms of 9 antioxidants are shown in Figure B.1 of Appendix B.

5.4.2 Quantitative determination

Inject the specimen solution and blank sample solution into the high-performance liquid chromatograph; obtain the peak area; and calculate the concentrations of the 9 antioxidants in the test solution based on the standard curve.

6. Expression of Analytical Results

6.1 Calculation of specific migration of antioxidants in non-sealed food contact materials and products (expressed in mg/kg)

For food contact materials and products other than lids, sealing rings, connectors and other sealing products (hereinafter referred to as sealing products), when the specific migration of antioxidants is expressed in mg/kg, it shall be calculated according to Formula (1).

$$X_1 = \frac{(c - c_0) \times V}{S} \times \frac{S_0}{m_1} \qquad \cdots\cdots(1)$$

Where:

X_1 – specific migration of antioxidant, in mg/kg;

c – antioxidant content in the specimen solution, in mg/L;

c_0 – antioxidant content in the blank test solution, in mg/L;

V – volume of specimen soaking solution, in L;

S – contact area between the specimen and soaking solution in the migration test, in dm^2;

S_0 – contact area between the non-sealing products in the actual use period and the food, in dm^2;

m_1 – mass of non-sealing products in actual contact with the solid food, or food mass corresponding to the volume of the actually-contacting liquid food, in kg; the volume of each liquid food with density of 1kg/L is converted into the corresponding mass.

The results shall be retained at least 2 significant figures.

6.2 Calculation of specific migration of antioxidants in sealed product food contact materials and products

6.2.1 When the intended use is known, for sealed product food contact materials and products, when the specific migration of antioxidants is expressed in mg/kg, it shall be calculated according to Formula (2).

$$X_2 = \frac{(c - c_0) \times V}{S} \times \frac{S_0}{m_2} \qquad \cdots\cdots(2)$$

Where:

X_2 – specific migration of antioxidant, in mg/kg;

c – antioxidant content in the specimen solution, in mg/L;

c_0 – antioxidant content in the blank test solution, in mg/L;

V – volume of specimen soaking solution, in L;

S – contact area between the specimen and soaking solution in the migration test, in dm^2;

S_0 – contact area between the non-sealing products in the actual use period and the food, in dm^2;

m_2 – mass of solid food contained in the actually-used container of the sealing products, or food mass corresponding to the volume of the actually-contacted liquid food, in kg; the volume of each liquid food with density of 1kg/L is converted into the corresponding mass.

The results shall be retained at least 2 significant figures.

6.2.2 When the intended use is unknown, for food contact materials and products of the sealed products, when the specific migration of antioxidants is expressed in mg/piece, it shall be

10.1.3 Ammonium fluoride (NH_4F).

10.1.4 Ethyl acetate ($C_4H_8O_2$).

10.2 Preparation of reagent

10.2.1 Ethanol-containing foods, acidic foods and chemical alternative solvents: Prepare according to the requirements of GB 5009.156.

10.2.2 Ammonium fluoride solution (1 mmol/L): Weigh 0.037g of ammonium fluoride in a beaker; add water to dissolve; and make constant volume of 1L in a volumetric flask; filter with suction and set aside.

10.3 Standard products

The same as 3.3.

10.4 Preparation of standard solution

10.4.1 Standard stock solutions of 9 antioxidants (1000 mg/L): The same as 3.4.1.

10.4.2 Standard intermediate solution C of 9 antioxidant (solvent is methanol): Accurately pipette 1.5mL of each antioxidant 264, 2,4-di-tert-butylphenol and antioxidant 168 standard stock solution; and pipette 0.50mL of other 6 antioxidants standard stock solution into the same 50mL brown volumetric flask; use the methanol to make constant volume to the scale; and obtain the standard intermediate solution C of antioxidant 264, 2,4-di-tert-butylphenol and antioxidant 168 with mass concentrations 30mg/L, and of the other 6 antioxidants with mass concentration 10mg/L. Transfer the solution to a brown glass container and store it in an airtight container away from light in a 4°C refrigerator. The storage period is 3 months.

10.4.3 Standard intermediate solution D of 9 antioxidants (solvent is ethyl acetate): Accurately pipette 1.5mL of each antioxidant 264, 2,4-di-tert-butylphenol and antioxidant 168 standard stock solutions; and pipette 0.50mL of other 6 antioxidant standard stock solution into the same 50 mL brown volumetric flask; use the ethyl acetate to make constant volume to obtain standard intermediate solution of antioxidant 264, 2,4-di-tert-butylphenol and antioxidant 168 with mass concentrations of 30 mg/L, and the other 6 antioxidants with mass concentration of 10 mg/L. Transfer the solution to a brown glass container and store it in a 4°C refrigerator in a dark and airtight container. The storage period is 3 months.

10.4.4 Preparation of standard working solution

10.4.4.1 Chemical alternative solvent isooctane standard working solution

Accurately draw 0.1mL, 0.5mL, 1mL, 3mL, and 5mL of 9 antioxidant standard intermediate solutions D into 5 pieces of 10mL volumetric flasks, respectively; use isooctane to make constant volume; and obtain standard working solution of antioxidants 264, 2,4-di-tert-butylphenol and antioxidant 168 with mass concentration of 0.30mg/L, 1.5mg/L, 3.0mg/L,

9mg/L, and 15mg/L respectively, and other 6 antioxidants with mass concentration of 0.10mg/L and 0.50mg/ L, 1.0mg/L, 30mg/L, 5.0mg/L standard working solutions. Respectively pipette 2.00mL of the above standard working solutions into 5 pieces of 10mL test tubes; blow with nitrogen at 40°C until almost dry; add 2.00mL of methanol to dissolve; vortex and redissolve; filter through a needle nylon filter; and wait for measurement on the machine.

10.4.4.2 Standard working solution of other food simulants and chemical alternative solvents 95% (volume fraction) ethanol

Accurately pipette 0.1mL, 0.5mL, 1mL, 3mL, and 5mL of the 9 antioxidant standard intermediate solutions C into 10mL volumetric flasks; use 10% ethanol to make constant volume; and obtain standard working solution of antioxidants 264, 2,4-di-tert-butylphenol and antioxidants 168 with mass fraction of 0.30 mg/L, 1.5 mg/L, 3.0 mg/L, 9 mg/L, and 15 mg/L, respectively; and of the other 6 antioxidants with mass concentration of 0.10 mg/L, 0.50 mg/L, and 1.0mg/L, 30mg/L [Translator Note: here it shall be 3.0mg/L], 5.0mg/L, respectively. Using the same method, prepare 9 antioxidant standard working solutions of the same concentration series with water, 4% acetic acid, 10% ethanol, 20% ethanol, 50% ethanol food simulant and chemical alternative solvent 95% ethanol. Pipette 1 mL of each standard working solution; filter it through a needle nylon filter; and wait for measurement on the machine.

11 Apparatus

11.1 Liquid chromatography-tandem mass spectrometer: Equipped with electrospray ion source (ESI).

11.2 Analytical balance: Sensitivity is 0.0001g.

11.3 Nitrogen blowing concentrator.

11.4 Glass micro-syringe: 10μL, 100μL and 1000μL.

11.5 Thermostatic equipment.

11.6 Vortex oscillator.

11.7 Needle nylon filter: Pore size is 0.22μm.

11.8 Suction filtration device.

12 Analytical Procedures

12.1 Preparation of test solution

The same as 5.1.

Determine the food simulant specimen solution and standard working solution according to the instrument reference conditions listed in 12.2. If the chromatographic peak retention time deviation of the food simulant specimen solution and the standard solution is within ±2.5%, and the relative abundance of the qualitative ion pair is consistent with that of the mixed matrix standard solution of equivalent concentration, and the relative abundance deviation does not exceed the provisions of Table 4, it shall be judged that the corresponding substance to be measured is present in the sample. The main reference mass spectrometry parameters of 9 antioxidants are shown in Appendix C. The liquid chromatography-mass spectrometry/mass spectrometry multiple reaction monitoring (MRM) chromatograms of the 9 antioxidants are shown in Figure D.1 of Appendix D.

12.4.2 Quantitative determination

Inject the specimen solution and blank specimen solution into the liquid chromatography-tandem mass spectrometer, respectively to obtain the peak area of the target substance to be measured; and calculate the concentrations of 9 antioxidants in the specimen solution based on the standard working curve.

13 Expression of Analytical Results

The same as Clause 6.

14 Precision

The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 15% of the arithmetic mean.

15 Others

When the S/V in the migration test is the same as the S/V in the actual use of the sample, the detection limit and quantitation limit of the migration of antioxidants in each simulant and chemical alternative solvent by this method are shown in Table 5. When the S/V in the migration test is different from the S/V in the actual use of the sample, the detection limit and quantitation limit of the migration of antioxidants in each simulant and chemical alternative solvent by this method is converted according to Clause 13.

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