



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 31604.58-2023

**National food safety standard - Food contact materials and
products - Determination of migration of nine antioxidants**

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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.

3.1.1 Foods containing ethanol, acidic food simulants and chemical alternative solvents. the

reagents used must comply with the provisions of GB 5009.156.

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 $\geq 98\%$, 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.

3.4.4.1 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.

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 degrees C;

e) Injection volume. 50uL.

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.

5.4.1 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 $\pm 2.5\%$, 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