



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

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**Determination of propyl gallate in oils and fats**

食品安全国家标准 食品中9种抗氧化剂的测定

**(English Translation)**

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## 1 Scope

This Standard specifies five methods - high-performance liquid chromatography, liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, gas chromatography, and colorimetry - for the determination of nine antioxidants in food. propyl gallate (PG), 2,4,5-trihydroxybutyrophenone (THBP), tert- butylhydroquinone (TBHQ), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), 2,6-di-tert-butyl-4-hydroxymethylphenol (IonoX-100), octyl gallate (OG), butylated hydroxytoluene (BHT), and dodecyl gallate (DG).

The liquid chromatography method specified in this Standard is applicable to the determination of PG, THBP, TBHQ, NDGA, BHA, BHT, IonoX-100, OG, and DG in foods. Liquid chromatography-tandem mass spectrometry is applicable to the determination of THBP, PG, OG, NDGA, and DG in foods. Gas chromatography-mass spectrometry is applicable to the determination of BHA, BHT, TBHQ, and IonoX-100 in foods. Gas chromatography is applicable to the determination of BHA, BHT, and

TBHQ in foods. Colorimetry is applicable to the determination of PG in oils and fats.

Method One -- High-performance liquid chromatography

## 2 Principle

Oil and fat samples are dissolved in an organic solvent and purified using gel permeation chromatography (GPC). Solid food samples are dissolved in n-hexane, extracted with acetonitrile, and purified using solid-phase extraction columns.

Determination is performed by high-performance liquid chromatography, with quantification based on the external standard method.

## 3 Reagents and materials

Unless otherwise specified, all reagents used in this method are chromatographically pure, and the water is Grade I water as specified in GB/T 6682.

### 3.1 Reagents

#### 3.1.1

Formic acid (HCOOH).

3.3.8 Tert-butylhydroquinone. purity  $\geq 98\%$ .

3.3.9 2,6-Di-tert-butyl-4-hydroxymethylphenol. purity  $\geq 98\%$ .

**NOTE:** See Annex A for English names, molecular formulas, and CAS numbers.

**3.4.1 Mixed stock solution of antioxidant reference materials. Accurately weigh 0.1 g**

(to the nearest 0.1 mg) of solid antioxidant reference materials. Dissolve the materials in acetonitrile in a 100 mL amber volumetric flask and dilute to the mark to prepare a mixed stock solution with a concentration of 1000 mg/L. Store at 0°C~4°C, protected from light.

**3.4.2 Antioxidant mixed working standard solutions. Pipette appropriate volumes of**

the mixed antioxidant standard stock solution (1000 mg/L) and dilute them to prepare mixed working standard solutions with concentrations of 20 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L, respectively.

**3.5 Materials**

**3.5.1**

C18 solid-phase extraction column. 2000 mg/12 mL.

**3.5.2**

Organic membrane filter. pore size is 0.22 µm.

**4 Instruments and equipment**

**4.1**

Centrifuge. rotational speed  $\geq 3000$  r/min.

**4.2**

Rotary evaporator.

**4.3**

High-performance liquid chromatograph.

**4.4**

Gel permeation chromatograph.

**4.5**

Analytical balance. resolution of 0.01 g and 0.1 mg.

**4.6**

Vortex mixer.

## 5.1 Specimen preparation

Pulverize and homogenize solid or semi-solid samples. Then, using the diagonal method, take two-quarters or two-sixths of the material, or otherwise obtain a representative sample based on the specific nature of the specimen. Seal and store. For liquid samples, mix thoroughly, take a representative sample, and seal for storage.

### 5.2.1 Extraction

#### 5.2.1.1

For solid samples. Weigh 1 g (to the nearest 0.01 g) of the specimen (5.1) into a 50 mL centrifuge tube. Add 5 mL of acetonitrile-saturated n-hexane. Vortex for 1 min to mix thoroughly, then let soak for 10 min. Add 5 mL of saturated sodium chloride solution. Vortex with 5 mL of n-hexane-saturated acetonitrile for 2 min and centrifuge at 3000 r/min for 5 min. Collect the acetonitrile layer in a test tube. Repeat the extraction twice more using 5 mL of n-hexane-saturated acetonitrile each time. Combine the three extracts. Adjust the pH to 4 using 0.1% formic acid solution, and set aside for cleanup.

Conduct a blank test in parallel.

#### 5.2.1.2

For oil samples. Weigh 1 g (to the nearest 0.01 g) of the specimen (5.1) into a 50 mL centrifuge tube. Add 5 mL of acetonitrile-saturated n-hexane to dissolve the sample. Vortex for 1 min and let stand for 10 min. Extract by vortexing for 2 min with 5 mL of n-hexane-saturated acetonitrile. Centrifuge at 3000 r/min for 5 min and collect the acetonitrile layer in a test tube. Repeat the extraction twice more using 5 mL of n-hexane-saturated acetonitrile each time. Combine the three extracts for subsequent cleanup. Conduct a blank test in parallel.

### 5.2.2 Purification

Pack a C18 solid-phase extraction (SPE) column with approximately 2 g of anhydrous sodium sulfate. Condition the column with 5 mL of methanol, then equilibrate it with 5 mL of acetonitrile. Discard the effluent. Pour the entire extract (from 5.2.1) into the column and discard the effluent. Elute with 5 mL of an acetonitrile-methanol mixture and collect the eluate in a test tube. Evaporate to dryness by rotary evaporation at 40°C.

Reconstitute the residue to a final volume of 2 mL with acetonitrile. Filter the solution through a 0.22 µm organic-compatible membrane filter prior to liquid chromatographic analysis.