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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 28316-2012

**National food safety standard - Food additive -
Lycopene**

食品安全国家标准

食品添加剂 番茄红

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National food safety standard - Food additive - Lycopene

1 Scope

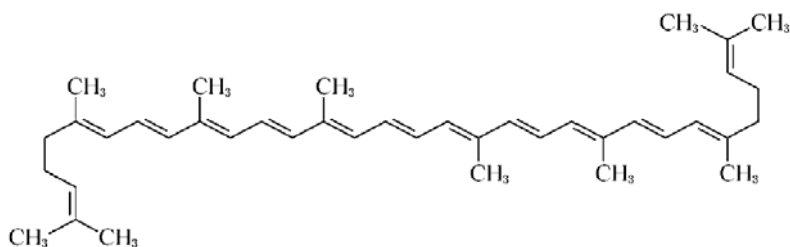
This standard applies to the food additive lycopene which is prepared by tomato (Lycopersicon) or tomato products as raw materials, using supercritical fluid (including carbon dioxide, etc.) or organic solvents as the extraction medium.

2 Molecular formula, structural formula, relative molecular mass

2.1 Molecular formula

Lycopene: $C_{40}H_{56}$

2.2 Structural formula



All-trans lycopene chemical structure

2.3 Relative molecular mass

536.87 (according to 2007 international relative atomic mass)

3 Technical requirements

3.1 Sensory requirements: It shall meet the requirements of Table 1.

Appendix A

Testing method

A.1 General provisions

The reagents and water used in this standard refer to analytical reagents and the level 3 water as specified in GB/T 6682-2008, unless otherwise specified. The standard titration solution, standard solution for impurity determination, preparations and products used in the test are all prepared in accordance with the requirements of GB/T 601, GB/T 602, GB/T 603, unless otherwise specified. The solution used in the test refers to an aqueous solution when it is not specified which solvent is used for preparation.

A.2 Identification test

A.2.1 Solubility

Easily soluble in ethyl acetate and n-hexane, partially soluble in ethanol and acetone, but insoluble in water.

A.2.2 Color reaction

Weigh about 0.01 g of the specimen and dissolve it in 10 mL of acetone, to form an orange-red transparent solution. Continuously add an equal volume of 5% sodium nitrate solution (5 g dissolved in 100 mL water) and 1 mol/L sulfuric acid solution. The color becomes lighter.

A.2.3 Characteristic absorption peak

Take an appropriate amount of specimen and dissolve it in n-hexane. This specimen solution has characteristic absorption peaks at wavelengths around 446 nm, 472 nm, 505 nm.

A.3 Determination of lycopene content

A.3.1 Reagents and materials

A.3.1.1 Dichloromethane: Chromatographically pure.

A.3.1.2 Acetonitrile: Chromatographically pure.

A.3.1.3 Ethyl acetate: Chromatographically pure.

A.3.1.4 Water: Level 1 water.

A.3.1.5 Ethanol.

A.3.1.6 Petroleum ether: Boiling range 60 °C ~ 90 °C.

A.3.1.7 2,6-Di-tert-butyl-p-cresol (BHT).

A.3.1.8 Lycopene reference substance: Purity $\geq$ 90%.

A.3.2 Instruments and equipment

A.3.2.1 High performance liquid chromatograph.

A.3.2.2 UV-Visible spectrophotometer.

A.3.3 Reference chromatographic conditions

A.3.3.1 Chromatographic column: The stationary phase is C₁₈, 250 mm × 4.6 mm, 5 μ m; or other equivalent chromatographic column.

A.3.3.2 Column temperature: 25 °C.

A.3.3.3 Detector: UV/visible light detector or diode array detector.

A.3.3.4 Detection wavelength: 472 nm.

A.3.3.5 Mobile phase: Mobile phase A is acetonitrile solution (9 + 1); mobile phase B is ethyl acetate. The elution condition is a binary linear gradient: Mobile phase B rises from 0% to 100% within 20 minutes; after 20 minutes, mobile phase B keeps running at 100% for 5 minutes.

A.3.3.6 Flow rate: 1.0 mL/min.

A.3.3.7 Injection volume: 10 μ L.

A.3.4 Analytical procedures

A.3.4.1 Preparation of BHT solution

Accurately weigh 2.5 g of BHT and place it in a 500 mL brown volumetric flask. Use dichloromethane to make its volume reach to the mark. The concentration is 5000 mg/L. Place it in a closed and dark place. It can be stored stably for 3 months.

A.3.4.2 Preparation of lycopene reference substance solution

Weigh 10 mg of lycopene reference substance and 20 mg of BHT, respectively, accurate to 0.000 1 g. Take a small amount of dichloromethane to dissolve. Transfer it into a 100 mL brown volumetric flask. Use dichloromethane to make its volume reach to the mark. Mix it uniformly. Because lycopene is unstable, the lycopene reference substance solution shall be calibrated for its purity by A.3.4.3 method before use. The lycopene reference substance solution shall be

determination results. The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 2% of the arithmetic mean.

A.5 Determination of ethyl acetate

A.5.1 Instruments and equipment

A gas chromatograph equipped with a headspace sampler and a flame ionization detector (FID).

A.5.2 Reference chromatographic conditions

A.5.2.1 Chromatographic column: Quartz capillary column cross-linked with 5% diphenyl to 95% polydimethylsiloxane fixing solution, 30 m × 0.53 mm, film thickness 3.0 μm; or other equivalent chromatographic columns column.

A.5.2.2 Carrier gas: Nitrogen.

A.5.2.3 Carrier gas flow: 4.0 mL/min.

A.5.2.4 Temperature of the sample inlet: 180 °C.

A.5.2.5 Column temperature: 73 °C, keep for 5 min; heat up to 160 °C at a rate of 25 °C/min, keep it for 1 min.

A.5.2.6 Detector temperature: 230 °C.

A.5.2.7 Injection volume: 1 mL quantitative ring.

A.5.2.8 Split ratio: 1:6.

A.5.3 Headspace conditions

A.5.3.1 Headspace bottle's equilibrium temperature and time: 70 °C, 120.0 min.

A.5.3.2 Quantitative ring's temperature: 100 °C.

A.5.3.3 Transmission line's temperature: 110 °C.

A.5.3.4 Mixing method: Stir for 1 min every 30 min.

A.5.3.5 Pressurization time: 0.2 min.

A.5.3.6 Filling time of quantitative ring: 0.2 min.

A.5.3.7 Quantitative ring's equilibrium time: 0.05 min.

A.5.3.8 Sample injection time: 1.0 min.

A.5.3.9 Headspace bottle's pressure: 95.15 kPa (13.8 psi).

A.5.3.10 Sample injection volume: 1 mL.

A.5.4 Analytical procedures

A.5.4.1 Preparation of control solution

A.5.4.1.1 Ethyl acetate stock solution A (10000 mg/kg)

Place an empty measuring bottle on the balance. After deducting the weight of the bottle, accurately add 500 mg of ethyl acetate. Then accurately add diethyl phthalate to make the solvent volume reach 50.00 g, accurate to 0.1 mg. Ultrasonically mix it uniformly. This solution can be stored stably for 2 months at room temperature.

A.5.4.1.2 Ethyl acetate stock solution B (100 mg/kg)

Place an empty measuring bottle on the balance. After deducting the weight of the bottle, accurately add 500 mg of ethyl acetate stock solution A. Then accurately add diethyl phthalate to make the solvent volume reach 50.00 g, accurate to 0.1 mg. Ultrasonically mix it uniformly. This solution can be stored stably for 2 months at room temperature.

A.5.4.1.3 Ethyl acetate standard solution C (5 mg/kg)

Place an empty measuring bottle on the balance. After deducting the weight of the bottle, accurately add 500 mg of ethyl acetate stock solution B. Then accurately add diethyl phthalate to make the solvent volume reach 10.00 g, accurate to 0.1 mg. Place in a 12 mm ~ 15 mm magnetic stir bar. Seal it. Mix it uniformly.

A.5.4.1.4 Ethyl acetate standard solution D (10 mg/kg)

Place an empty measuring bottle on the balance. After deducting the weight of the bottle, accurately add 1000 mg of ethyl acetate stock solution B. Then accurately add diethyl phthalate to make the solvent volume reach 10.00 g, accurate to 0.1 mg. Place in a 12 mm ~ 15 mm magnetic stir bar. Seal it. Mix it uniformly.

A.5.4.1.5 Ethyl acetate standard solution E (17.5 mg/kg)

Place an empty measuring bottle on the balance. After deducting the weight of the bottle, accurately add 1750 mg of ethyl acetate stock solution B. Then accurately add diethyl phthalate to make the solvent volume reach 10.00 g, accurate to 0.1 mg. Place in a 12 mm ~ 15 mm magnetic stir bar. Seal it. Mix it uniformly.

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