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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 1886.78-2016

**National Food Safety Standard -
Food Additives - Lycopene (synthetic)**

食品安全国家标准

食品添加剂 番茄红素（合成）

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National Food Safety Standard - Food Additives - Lycopene (synthetic)

1 Scope

This Standard is applicable to the food additive lycopene prepared by Wittig condensation reaction using synthetic intermediates commonly used in the production of other carotenoids in foods as the raw materials.

2 Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Molecular Formula

$C_{40}H_{56}$

2.2 Structural Formula

2.3 Relative Molecular Mass

536.87 (in accordance with 2007 international relative atomic mass)

3 Technical Requirements

3.1 Sensory Requirements

Sensory requirements shall comply with the stipulations of Table 1.

Appendix A

Inspection Method

A.1 General Rules

When other requirements are not indicated, the reagents and water used in this Standard refer to analytically pure reagents and Grade-3 water specified in GB/T 6682. When other requirements are not indicated, the standard solutions used in the reagents, and the standard solutions, preparations and products used for impurity determination shall be prepared in accordance with the stipulations of GB/T 601, GB/T 602 and GB/T 603. When it is not specified which solvent is used for the preparation, the solutions used in the test refer to aqueous solutions.

A.2 Identification Test

A.2.1 Almost insoluble in water; soluble in organic solvents, for example, chloroform.

A.2.2 Sensitive to heat, light, air and humidity; easily oxidized.

A.3 Determination of Total Lycopene Content and All-trans-lycopene Content

A.3.1 Method summary

Under specified conditions, use high-performance liquid chromatography to determine the total lycopene content and all-trans-lycopene content.

A.3.2 Reagents and materials

A.3.2.1 N-hexane: chromatographically pure.

A.3.2.2 Tetrahydrofuran stabilized with 0.025% tert-butylhydroquinone.

A.3.2.3 N-ethyl-diisopropylamine: chromatographically pure.

A.3.2.4 Lycopene standard substance: purity $\geq 95\%$.

A.3.3 Instruments and equipment

A.3.3.1 UV / visible spectrophotometer, equipped with 1 cm absorption cell.

A.3.3.2 High-performance liquid chromatography system, equipped with suitable pump, sample injector, chromatographic column thermostat and integrator.

A.3.3.3 Chromatographic column: 2 connected stainless-steel columns (250.0 mm $\times$ 4.0 mm).

A.3.3.4 Stationary phase: Nucleosil 300-5, 5 µm.

A.3.3.5 Reference chromatographic conditions:

- a) Flow rate: 0.8 mL/min;
- b) Sample injection volume: 20 µL;
- c) Column temperature: 20 °C;
- d) Detection wavelength: 470 nm;
- e) Mobile phase: 0.15% (volume fraction) N-ethyl-diisopropylamine in N-hexane solution.

A.3.3.6 Standard solution for high-performance liquid chromatography determination: accurately weigh-take 5.5 mg ~ 6.5 mg of lycopene standard substance, place it in a 100 mL measuring flask; add 5 mL of tetrahydrofuran stabilized with tert-butylhydroquinone to dissolve it; use N-hexane to dilute to the scale. It shall be considered as a standard solution for high-performance liquid chromatography determination.

A.3.3.7 Standard solution for spectrophotometric determination: accurately weigh-take 5.0 mL of the standard solution (A.3.3.6) for high-performance liquid chromatography determination; place it in a 100 mL measuring flask; use N-hexane to dilute to the scale. It shall be considered as a standard solution for spectrophotometric determination.

A.3.3.8 Sample solution: accurately weigh-take 4.5 mg ~ 5.5 mg of sample; place it in a 100 mL measuring flask; add 5 mL of tetrahydrofuran stabilized with tert-butylhydroquinone to dissolve it; use N-hexane to dilute to the scale.

A.3.4 Analytical procedures and result calculation

A.3.4.1 Determination of lycopene with spectrophotometry

A.3.4.1.1 Analytical procedures

Use 1 cm absorption cell; take N-hexane as the blank; at the maximum absorption wavelength (approximately 470 nm), determine the absorbance of the standard solution (A.3.3.7) used for spectrophotometric determination.

A.3.4.1.2 Result calculation

The concentration c_{st} of lycopene in the standard solution, expressed in (mg/L), shall be calculated in accordance with Formula (A.1):

$$c_{st} = \frac{A \times 10\,000}{3\,450} \dots\dots\dots (A.1)$$

A.4.4.1 Preparation of standard solution

Accurately weigh-take 14.5 mg ~ 15.5 mg of Apo-12'-lycopene aldehyde reference substance; place it in a 50 mL measuring flask. Use toluene stabilized with tert-butylhydroquinone to dissolve and dilute it to the scale. Accurately weigh-take 2 mL; place it in a 100 mL measuring flask; use toluene stabilized with tert-butylhydroquinone to dilute to the scale.

A.4.4.2 Preparation of sample solution

Accurately weigh-take 29.0 mg ~ 31.0 mg of sample; place it in a 10 mL measuring flask. Use toluene stabilized with tert-butylhydroquinone to dissolve and dilute it to the scale; conduct ultrasonic treatment for 10 min.

A.4.5 Result calculation

The retention time of apo-12'-lycopene aldehyde is approximately 14 min; its relative retention time relative to all-trans-lycopene is 1.6 min. The mass fraction w_3 of apo-12'-lycopene aldehyde shall be calculated in accordance with Formula (A.5):

$$w_3 = \frac{A_s \times W_{st} \times 10}{A_{st} \times W_s \times 2\,500} \times 100\% \quad \dots\dots\dots (A.5)$$

Where,

A_s ---peak area of sample;

W_{st} ---sampling size of standard substance, expressed in (mg);

10---volume of dissolved sample, expressed in (mL);

A_{st} ---peak area of standard substance;

W_s ---sampling size, expressed in (mg);

2,500---volume of dissolved reference substance (50 mL) multiplied by the dilution factor (50).

A.5 Determination of Triphenylphosphine Oxide (TPPO)**A.5.1 Method summary**

Under specified conditions, use high-performance liquid chromatography for detection.

A.5.2 Reagents and materials (all reagents shall all be chromatographic grade)

A.5.2.1 N-hexane.

A.5.2.2 Isopropanol.

A.5.2.3 Tetrahydrofuran.

A.5.2.4 Triphenylphosphine oxide (TPPO) (purity $\geq 99\%$).

A.5.3 Instruments and equipment

A.5.3.1 High-performance liquid chromatography system, equipped with suitable pump, sample injector and integrator.

A.5.3.2 Chromatographic column: stainless-steel column (150.0 mm $\times$ 4.6 mm).

A.5.3.3 Stationary phase: silica gel base column Supelcosil Lc-Si, 5 μm or similar column.

A.5.3.4 Detector: UV detector.

A.5.3.5 Conditions of high-performance liquid chromatography:

- a) Column temperature: 20 $^{\circ}\text{C}$;
- b) Mobile phase: isopropanol: N-hexane (volume ratio 1 : 24);
- c) Flow rate: 1.5 mL/min;
- d) Sample injection volume: 50 μL ;
- e) Detection wavelength: 210 nm;
- f) Retention time of TPPO is approximately 8.1 min.

A.5.4 Analytical procedures

Standard solution: accurately weigh-take 10 mg of TPPO standard substance; use tetrahydrofuran to dissolve it, then, quantitatively transfer it into a 1,000 mL volumetric flask; use tetrahydrofuran to dilute to the scale.

Sample solution: accurately weigh-take 1,000 mg of sample; use tetrahydrofuran to dissolve it, then, quantitatively transfer it into a 100 mL volumetric flask; use tetrahydrofuran to dilute to the scale.

A.5.5 Result calculation

Mass fraction w_4 of triphenylphosphine oxide (TPPO) shall be calculated in accordance with Formula (A.6):

$$w_4 = \frac{A_s \times W_{st} \times P_{st} \times 100}{A_{st} \times m_s \times 1\,000} \times 100\% \dots\dots\dots (A.6)$$

Where,

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