



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

GB 26405-2011

National food safety standard - Food additives - Lutein

食品添加剂 叶黄素

(English Translation)

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Table of Contents

1 Scope	3
2 Molecular formula, structural formula and relative molecular mass	3
3 Technical requirements	3
Appendix A Inspection methods	5

1 Scope

This Standard applies to the food additive lutein, which is made from *Tagetes erecta* L. oleoresin through saponification and extraction. Commercial lutein products can contain edible vegetable oils, dextrins, antioxidants and other auxiliary materials, which are used for standardization and other purposes.

2 Molecular formula, structural formula and relative molecular mass

2.1 Molecular formula

C₄₀H₅₆O₂

2.3 Relative molecular mass

568.88 (according to the international relative atomic mass in 2007)

3.1 Sensory requirements

shall meet the requirements of Table 1.

3.2 Physical and chemical indicators

shall meet the requirements of Table

2.

Appendix A

Inspection methods

A.1 General provisions

The reagents and water that are used in this Standard, when no other requirements are specified, refer to analytical reagents and grade-III water which is specified in GB/T 6682-2008. The standard titration solution which are used in the test, the standard solutions

for impurity determination, the preparations and products, are all prepared in accordance with the provisions of GB/T 601, GB/T 602, and GB/T 603, when no other requirements are specified. The solution used in the test, if not indicated which solvent is used, refers to aqueous solution.

A.2 Identification test

A.2.1 Insoluble in water, extremely-slightly soluble in n-hexane, soluble in ethanol and chloroform.

A.2.2 In the test for determining the total carotenoid content, the sample solution has a maximum absorption near 446 nm.

A.2.3 In the test for determining the lutein content, the retention time of the main peak of the sample solution in the liquid chromatogram shall be the same as the retention time of lutein in the standard solution.

A.3.1.1 Solvent

a mixture of n-hexane, acetone, toluene and absolute ethanol (10:7:7:6).

A.3.1.2 Absolute ethanol.

A.3.2 Instruments and apparatuses

Ultraviolet-visible spectrophotometer.

A.3.3 Analysis steps

Accurately weigh 0.03 g of the sample, accurate to 0.000 1 g; use the solvent of A.3.1.1 to dissolve it; transfer to a 100 mL volumetric flask; add the solvent of A.3.1.1 to fix volume to the mark; shake well. Take 1 mL of this sample solution in a 100 mL volumetric flask; use absolute ethanol to fix volume to the

A.4.3.1 Chromatographic column

silica gel column, 4.6 mm x 250 mm, particle size of 3 µm; or other equivalent chromatographic column.

A.4.3.2 Mobile phase

prepare according to the volume ratio, that is, n-hexane:

ethyl acetate = 70: 30; after mixing uniformly, use a 0.45 µm filter membrane for filtration; perform ultrasonic degassing for later use.

A.4.3.3 Column temperature

room temperature.

A.4.3.4 Mobile phase flow rate

1.5 mL/min.

A.4.3.5 Injection volume

10 μ L.

A.4.4.1 Preparation of standard solution

Respectively weigh about 0.01 g of lutein and zeaxanthin standard substances, accurate to 0.000 1 g; use mobile phase to dissolve; transfer to a 50 mL volumetric flask; add mobile phase to fix volume to the mark; shake well for later use.

A.4.4.2 Preparation of sample solution

Weigh 0.025 g ~ 0.027 g of the sample, accurate to 0.000 1 g; use mobile phase to dissolve it; transfer it into a 100 mL volumetric flask; add mobile phase to fix volume to the mark; shake well for later use.

A.4.4.3 Determination

Under the reference chromatographic conditions of A.4.3, determine the standard solution of lutein and zeaxanthin; record the chromatogram. It is required that the number of theoretical plates shall be at least 5 000 based on the lutein peak, and the separation between the main peak and the isomer peak shall be at least 1.5.

Under the reference chromatographic conditions of A.4.3, measure the sample solution; determine the chemical composition based on the retention time of the standard; record the chromatogram. Repeat the experiment twice to get the average peak area values of lutein and zeaxanthin respectively.

A.4.5 Result calculation

The lutein content is calculated as the mass fraction w_1 of lutein; the value is expressed in %, and calculated according to Formula (A.2):

A.5.3.1 Headspace bottle temperature

80 °C.

A.5.3.2 Quantitative loop temperature

85 °C.

A.5.3.3 Transmission line temperature

100 °C.

A.5.3.4 Headspace bottle equilibrium time

40.0 min.

A.5.3.5 Gas cycle time

30.0 min.

A.5.3.6 Pressurization time

0.2 min.

A.5.3.7 Quantitative loop filling time

0.2 min.

A.5.3.8 Quantitative loop equilibrium time

0.05 min.

A.5.3.9 Sampling time

1.0 min.

A.5.3.10 Headspace bottle pressure

95.15 kPa.

A.5.4.1 Preparation of control solution

Precisely draw 1.5 µL of n-hexane in a 100 mL volumetric flask; use dimethylformamide (DMF) to dilute to the mark; shake well; draw 25.0 mL to a

50 mL volumetric flask; use DMF to fix volume. This is the control solution (in the control solution, the alkane concentration is 0.004 95 mg/mL).

Sensitivity solution preparation: Draw 1.0 mL of the control solution into a 10 mL volumetric flask; use DMF to fix volume; shake well (the concentration of n- hexane in the sensitivity solution is 0.000 495 mg/mL).