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Food Additive Lutein

Report Categories:

FAIRS Subject Report

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Report Highlights:

On May 2, China's Ministry of Health notified to the WTO the National Food Safety Standard on Food Additive Lutein as G/SPS/N/CHN/359. The standard applies to lutein obtained from marigold (Tages erecta L.). It specifies the technical requirements and testing methods for food additive lutein. The adoption date of the standard is May 15, 2011. This report contains an INFORMAL translation of the document.

General Information:
BEGIN TRANSLATION

National food safety standard

Food Additive Lutein

GB26405-2011

Issued on March 15, 2011

Implemented on May 15, 2011

Issued by the Ministry of Health

National Food Safety Standard
Food Additive
Lutein

1. Scope

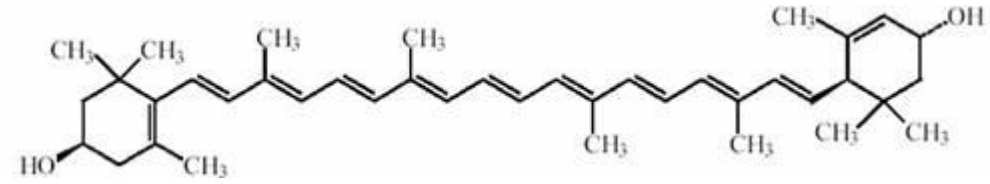
This Standard is applicable for lutein, a kind of food additive using marigold (Tageteserecta L.) oleoresin as raw materials and produced through procedures including saponification and extraction and purification. Commercialized lutein products are allowed to contain edible vegetable oil, dextrin, antioxidants, etc. which are for the purpose of standardization.

2. Molecular Formula, Constitutional Formula and Relative Molecular Mass

2.1 Molecular Formula

C40H56O2

2.2 Constitutional Formula



2.3 Relative Molecular Mass

568.88(as per 2007 International Relative Molecular Mass)

3 Technical Requirements

3.1 Sensory Requirements shall comply to the requirements in Table 1.

Table 1 Sensory Requirements

Item	Requirements	Testing Method
Color	Orange yellow to orange red	Put an appropriate amount of samples into a white clean and dry porcelain plate, and observe the color and status under natural light.
Tissue Status	Powder	

3.2 Physicochemical Indicators shall comply to the requirements in Table 2.

Table 2 Physicochemical Indicators

Item	Indicators	Testing Method

Total Carotenoids, *w*/%	≥	80.0	A.3, Appendix A
Lutein, *w*/%	≥	70.0	A.4, Appendix A
Zeaxanthin, *w*/%	≤	9.0	A.4, Appendix A
Loss on Drying, *w*/%	≤	1.0	GB 5009.3 Depressurized Drying Method
Ash Content, *w*/%	≤	1.0	GB 5009.4
N-hexane /(mg/kg)	≤	50	A.5, Appendix A
Lead (Pb)/(mg/kg)	≤	3	GB 5009.12
Total Arsenic (Calculated as As) (mg/kg)	≤	3	GB/T 5009.11

Appendix A
Testing Method

A.1 General

Unless otherwise specified, the reagents and water in this standard all refer to analytical reagents and grade-III water provided in GB/T 6682—2008. Unless otherwise specified, the standard volumetric solutions used in the test, the standard solution, preparations and products for impurity test all are prepared according to the requirements in GB/T 601, GB/T 602, GB/T 603. The solutions used in the test all refer to water solution if the solvent for preparation is not specified.

A.2 Identification Test

A.2.1 Insoluble in water; very slightly soluble in n-hexane; soluble in ethanol and chloroform.

A.2.2 In the test of determining the content of total carotenoids, sample solution has maximum absorption around 446nm.

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

A.3 Determination of Total Carotenoids

A.3.1 Reagents and Materials

A.3.1.1 Solvent: N-hexane, acetone, and mixture of toluene and ethanol (10:7:7:6).

A.3.1.2 Absolute ethyl alcohol

A.3.2 Instruments and Equipment

UV - visible spectrophotometer

A.3.3 Analysis Steps

Weigh 0.03g test sample accurately, accurate to 0.0001g. Dissolve with A.3.1.1 solvent, transfer it to 100mL volumetric flask; add A.3.1.1 solvent and meter volume to the graduation and then shake. Take 1mL of this test sample solution into a 100mL volumetric flask, meter volume to the graduation with absolute ethyl alcohol. Place it into a 1cm cuvette, and do blank contrast with absolute ethyl alcohol. Test absorbency at 446nm±1nm with the maximum wavelength absorption with a UV - visible spectrophotometer. (Absorbency shall be between 0.3~0.7, otherwise the concentration of test sample shall be adjusted before retesting absorbency.)

A.3.4 Results Calculation

The content of total carotenoids is calculated as its mass fraction w_0 in %, and calculated with formula (A.1)

$$w_0 = \frac{A}{c} \times \frac{1}{2550} \quad \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \quad (A.1)$$

Where,

A—Actually determined absorbency of sample solution;

c—Concentration value of the determined sample solution, g/mL;

2550──Absorption coefficient of 1% sample solution at wavelength of 446nm in absolute ethyl alcohol.

The test result is subject to the arithmetic mean of results of parallel determination. The absolute difference of two separate determined results obtained repeatedly is not larger than 1.5% of arithmetic mean. One significant figure after the decimal point shall be kept in calculated results.

A.4 Determination of Lutein and Zeaxanthin

A.4.1 Reagents and Materials

A.4.1.1 N-hexane: Chromatography pure

A.4.1.2 Ethyl acetate: Chromatography pure

A.4.1.3 Lutein Standard products: Known purity

A.4.1.4 Zeaxanthin standard products: Known purity

A.4.2 Instruments and Equipment

High Performance Liquid Chromatography (HPLC) (The wavelength of detector is 446nm).

A.4.3 Reference Chromatographic Conditions

A.4.3.1 Chromatographic column: Silica gel column, 4.6mm*250mm, granularity 3μm; or other equivalent chromatographic column.

A.4.3.2 Mobile phase: Prepare with the ratio of n-hexane: Ethyl acetate= 70:30 (volume ratio). After mixing even, filter with 0.45μm filter membrane and prepare for use after ultrasonic degassing.

A.4.3.3 Column temperature: Room temperature

A.4.3.4 Flow rate of mobile phase: 1.5 mL/min

A.4.3.5 Sample injection volume: 10 μL

A.4.4 Analysis Procedure

A.4.4.1 Preparation of Standard Solution

Weigh about 0.01g lutein standard and zeaxanthin standard, accurate to 0.0001g, dissolve with mobile phase, and transfer to a 50mL volumetric flask, add mobile phase and meter volume to the graduation and then shake up for use.

A.4.4.2 Preparation of Sample Solution

Weigh about 0.025g~0.027g sample, accurate to 0.0001g, dissolve with mobile phase, and transfer to a 100mL volumetric flask, add mobile phase and meter volume to the graduation and then shake up for use.

A.4.4.3 Determination

Under A.4.3 reference chromatographic conditions, determine the standard sample solution of lutein and zeaxanthin, and record chromatogram map. Calculate by lutein peak; theoretical plate number is at least 5000. Separation of the main peak and isomer peak is at least 15.

Under A.4.3 reference chromatographic conditions, determine the sample solution, and record chromatogram map and determine the nature according to retention time of standards. Repeat the experiment twice, and obtain the value of average peak area of lutein and zeaxanthin.

A.4.5 Result Calculation

The content of lutein is calculated by its mass fraction w_1 in %, and calculated with Formula (A.2):

$$W_1 = W_0 \times P_1 \cdots\cdots (A.2)$$

The content of zeaxanthin is calculated by its mass fraction $w2$ in %, and calculated with Formula (A.3):

$$W_2 = W_0 \times P_2 \cdots\cdots (A.3)$$

Where,

w_0──Mass fraction of total carotenoids determined according to this Standard A.3, %.

P_1──Peak area percentage of lutein;

P_2 ── Peak area percentage of zeaxanthin

The actual result is subject to the arithmetic mean of results of parallel determination. The absolute difference of two separate determined results obtained repeatedly is not larger than 2% of arithmetic mean. One significant figure after the decimal point shall be kept in calculated results.

A.5 Determination of N-hexane

A.5.1 Instruments and Equipment
Gas chromatograph (GC), flame ionization detector (FID) and headspace sampler.
A.5.2 Reference Chromatographic Conditions
A.5.2.1 Chromatographic column: DB-624 capillary column, 30m*0.53mm, film thickness 3.0μm; or other equivalent chromatographic column.
A.5.2.2 Carrier gas: Helium gas or nitrogen gas
A.5.2.3 Flow rate of carrier gas: 3.0 mL/min
A.5.2.4 Temperature of sample inlet: 220 ℃
A.5.2.5 Column temperature: Keep 40 ℃ for 3min, then rise to 65 ℃ at 3.5 ℃ / min, and finally rise to 220 ℃ at 20 ℃ / min and keep for 5 min.
A.5.2.6 Detector temperature: 235 ℃
A.5.2.7 Sample volume: 1mL loop
A.5.2.8 Split ratio: 3:1
A.5.3 Headspace Conditions
A.5.3.1 Temperature of headspace vial: 80 ℃
A.5.3.2 Loop temperature: 85 ℃
A.5.3.3 Transmission line temperature: 100 ℃
A.5.3.4 Balance time of headspace vial: 40.0min
A.5.3.5 Cycle time of gas phase: 30.0min
A.5.3.6 Pressurizing time: 0.2min
A.5.3.7 Filling time of loop: 0.2min
A.5.3.8 Balance time of loop: 0.05min
A.5.3.9 Sample injection time: 1.0min
A.5.3.10 Headspace vial pressure: 95.15kPa
A.5.4 Analysis Procedure
A.5.4.1Preparation of Contrast Solution
Absorb precisely 1.5μL n-hexane into a 100mL volumetric flask, dilute with DMF to the graduation and shake up. Absorb 25.0 mL into a 50mL volumetric flask. Meter the volume wth DMF. This is contrast solution (Concentration of n-hexane in this contrast solution is 0.00495mg/mL).
Preparation of sensitivity solution: Absorb 1.0 mL contrast solution to 10 mL volumetric flask. Meter the volume with DMF and shake up (Concentration of n-hexane in this sensitivity solution is 0.000495 mg/mL).

A.5.4.2 Preparation of Sample Solution
Weigh precisely about 300mg test sample and place it into a 10mL headspace vial. Dilute with 3.0 mL DMF and shake up.
A.5.4.3 Requirements of System Suitability
Take 3.0 mL contrast solution mentioned in A.5.4.1 into a 10 mL headspace vial by 6 times continuously. Theoretical plate number of the main peak shall be larger than 5000. Peak area RSD shall not be larger than 10.0%. SNR of the main peak in sensitivity solution shall be larger than 3.
A.5.5 Result Calculation
Calculate n-hexane in the sample with the External Standard Method. The residue of n-hexane is calculated as mass fraction w_3 in mg/kg, and calculated with Formula (A.4):

$$w_3 = \frac{A_X \times C_R}{A_R \times C_X} \times 10^6 \quad \text{(A.4)}$$

Where,

A_X—Peak area value of n-hexane in sample solution;

C_R— Concentration value of n-hexane in contrast solution, mg/mL;

A_R—Peak area value of n-hexane in contrast solution;

C_X—Concentration value of sample solution, mg/mL.

END TRANSLATION