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LUTEIN FROM TAGETES ERECTA

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LUTEIN FROM TAGETES ERECTA

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP52 Add 12 (2004). A group ADI of "not specified" was established for Tagetes extract, Lutein from Tagetes erecta, Lutein esters from Tagetes erecta, Zeaxanthin (synthetic), and meso-zeaxanthin at the 86th JECFA (2018) superseding the group ADI of 0 - 2 mg/kg bw for lutein from T. erecta L. and synthetic zeaxanthin established at the 63rd JECFA (2004).

SYNONYMS INS No. 161b(i), Vegetable lutein; vegetable luteol; Bo-Xan, luteine

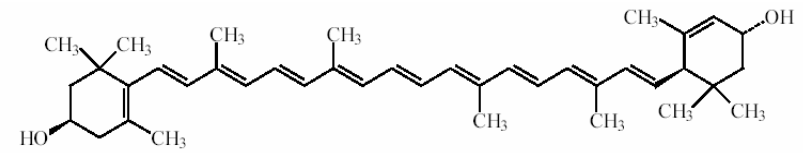
DEFINITION Lutein from *Tagetes erecta* is a purified extract of xanthophylls obtained from oleoresin in marigold. The oleoresin is prepared from hexane extracts of *Tagetes erecta* L. flowers, saponified with potassium hydroxide in either methanol or propylene glycol. The resulting reaction mixture is diluted with water and dried. The crystalline product contains lutein along with minor components that include other carotenoids and waxes.

Chemical names 3R,3'R,6'R-β,ε-carotene-3,3'-diol; all-*trans*-lutein; 4',5'-didehydro-5',6'-dihydro-beta,beta-carotene-3,3'-diol

C.A.S. number 127-40-2

Chemical formula $C_{40}H_{56}O_2$

Structural formula



Formula weight 568.88

Assay Not less than 80% total carotenoids, not less than 70% lutein

DESCRIPTION A free-flowing, orange-red powder

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, soluble in hexane
Spectrophotometry (Vol. 4)	A 2 mg/l solution in acetone shows maximum absorbance at approximately 446 nm.
Test for carotenoids (Vol. 4)	The colour of 2 ml of a 2 – 4 mg/l solution of the sample in acetone immediately disappears after successive addition of about 0.5 ml of 5% sodium nitrite and about 0.5 ml of 0.5 M sulfuric acid.

PURITY

Moisture (Vol. 4)	Not more than 1.0%
Ash (Vol. 4)	Not more than 1.0%
Zeaxanthin	Not more than 9.0% See description under METHOD OF ASSAY
Lead (Vol. 4)	Not more than 3 mg/kg. Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Hexane (Vol. 4)	Not more than 50 mg/kg
Methanol (Vol. 4)	Not more than 10 mg/kg
Propylene glycol	Not more than 1000 mg/kg See description under TESTS
Waxes	Not more than 14.0% See description under TESTS

TESTS

PURITY TESTS

Propylene glycol

Determine by gas chromatography (Vol. 4) under the following conditions.

Internal standard solution

Prepare a 500 µg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions

Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 µg/ml of propylene glycol and 5 µg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions

- Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
- Carrier gas: Helium
- Flow rate: 1.5 ml/min (Constant flow)
- Detector: FID
- Temperatures: injection port: 230°
- Column Temperature: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- Detector Temperature: 270°

The retention times of ethylene glycol and propylene glycol derivatives under the above conditions are approx. 7.6 min and 7.8 min, respectively.

Procedure

Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 µl of the internal standard solution. Dissolve and make up to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 µl of this centrifugal supernatant into the chromatograph.

Standard curve

Prepare following the same procedure using 0.5 ml of the

standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:

$$\mathrm{CPG\ (mg/kg)} = \mathrm{C} \times 10 / \mathrm{W}$$

where
C is polyethylene glycol concentration determined (µg/ml); and
W is weight of sample (g)

Waxes

Determine by gas chromatography (Vol. 4) using the following conditions:

- GC column DB-5 (30 m x 0.25 mm ID with a 0.25 µm film thickness) or equivalent.
- GC injector temperature: 280°
- FID temperature: 350°
- GC temperature program: 50° (2 min) 13°/min to 340° and hold for 8 min
- Carrier gas (Helium) flow rate: 1.0 ml/min
- Injection mode: splitless
- Injection volume: 1.0 µl

Standards:

- Hydrocarbons mixed standard: C25 to C46
- Internal standard: Hexatriancontane (C36)

Standard solutions:

Prepare standard solutions by addition of hydrocarbon standards to methylene chloride to get hydrocarbon concentrations of 2.0, 5.0, 10, 25, 50, mg/l respectively. Add required quantity of hexatriancontane internal standard to get a final concentration 50 mg/l in all standard solutions.

Sample Preparation

Accurately weigh 100 mg of sample into a centrifuge tube and dissolve in exactly 20 ml of methylene chloride. Sonication or vortex mixing may be required to completely dissolve the product. Centrifuge sample at 2500 rpm for 5 min, if the sample appears turbid. Add 1.6 ml of methylene chloride and 20 µl of (5000 mg/l) hexatriancontane solution (to a final concentration of 50 mg/l) into 2 ml volumetric flask. Transfer 40 µl of sample solution and dilute with methylene chloride to the 2 ml. Transfer the solution into a 2 ml autosampler vial.

Analysis

Inject 1.0 µl of each of the standards solutions. Record the peak areas. Construct standard curves using the peak ratios of each hydrocarbon to the internal standard against the concentration of the hydrocarbon. Inject 1.0 µl of the sample solution and determine individual wax in the sample (mg/l) from the respective standard curve. Add the concentration of individual waxes to get the total wax concentration in the sample solution (mg/l)

Calculation:

$$\text{Waxes \% w/w} = \frac{\text{C (mg/l)} \times 2\text{ ml} \times 20\text{ ml} \times 100}{1000\text{ (ml/l)} \times \text{W (mg)} \times 0.04\text{ ml}} = (100 \times \text{C})/\text{W}$$

Where:
C is the total concentration of waxes, mg/l in the sample
W is the weight of sample, mg

METHOD OF ASSAY

Determine the total carotenoid content and the content of lutein and zeaxanthin by UV spectrophotometry and HPLC using the following conditions:

Reagents:

- Hexane (HPLC grade)
- Ethyl acetate (HPLC grade)
- Acetone
- Dehydrated ethyl alcohol (absolute alcohol)
- Toluene
- Solvent Mixture: (10:6:7:7 hexane:dehydrated ethyl alcohol:acetone:toluene, v/v/v/v)

System Suitability Solution for HPLC:

150 µg/ml of lutein standard in solvent mixture (use USP Lutein RS available from U. S. Pharmacopeia, or equivalent standard)

Apparatus

UV/Vis spectrophotometer; 1-cm cuvettes
HPLC system with suitable diode array detector, autosampler, column oven, signal processor and degasser.
Analytical column: 3 µm silica, 4.6 mm x 250 mm

Instrument Conditions

- Temperature: ambient
- Mobile Phase: 70:30 (v:v) hexane/ethyl acetate (isocratic elution)
- Flow Rate: 1.5 ml/min
- Injection: 10 µl

- Detection: UV/Vis 446 nm
- Run Time: approximately 40 min

Concentrated Sample Preparation

For the UV/Vis spectrophotometry weigh sample (30 mg) into a glass weighing funnel. Using the solvent mixture, wash crystals into a 100 ml volumetric flask, dilute to the mark with the solvent mixture and stir for 10 min.

Sample Preparation

Pipette 1 ml of concentrated sample preparation into a 100 ml volumetric flask. Dilute up to the mark with dehydrated ethyl alcohol, mix by inversion for 20 seconds. Read samples in a spectrophotometer at 446 nm using dehydrated ethyl alcohol as the blank.

For HPLC, evaporate 1 ml of the concentrated sample preparation to dryness using a stream of nitrogen, dissolve solids in 1 ml 70:30 hexane:ethyl acetate, and add 0.5 ml to HPLC vials. Analyze this sample and the system suitability solution for HPLC using the HPLC conditions above.

Results

Compare the results of the chromatogram from the system suitability solution for HPLC to identify the lutein and zeaxanthin peaks at a resolution of not less than 3.

Calculation

Using the results obtained from the UV/Vis spectrophotometry calculate the % Total carotenoids

$$\%\ \text{Total carotenoids} = \frac{\text{Absorbance at 446 nm} \times 10000 \times 100}{\text{sample weight in g} \times 2550}$$

Note: The factors 10000 and 2550 are the dilution factor and extinction value for a 1% solution, respectively.

Using the chromatogram of the sample, calculate the concentration of lutein and zeaxanthin.

$$Lutein\ (\%) = \%\ Total\ carotenoids \times \frac{Peak\ Area\ LUTEIN}{Peak\ Area\ TOTAL}$$

$$Zeaxanthin\ (\%) = \%\ Total\ carotenoids \times \frac{Peak\ Area\ ZEAXANTHIN}{Peak\ Area\ TOTAL}$$