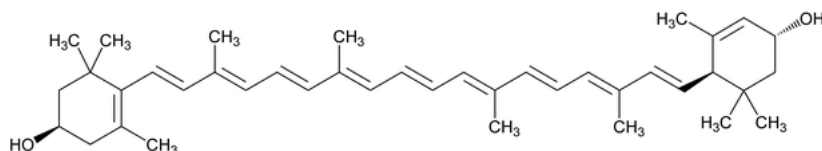


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Lutein



$C_{40}H_{56}O_2$ 568.87

β-ε-Carotene,3,3'-diol (3*R*,3'*R*,6'*R*) CAS RN[®]: 127-40-2.

DEFINITION

Lutein is the purified fraction obtained from saponification of the oleoresin of *Tagetes erecta* L. It contains NLT 80.0% of total carotenoids calculated as lutein ($C_{40}H_{56}O_2$). It contains NLT 74.0% of lutein and NMT 8.5% of zeaxanthin, both calculated as lutein ($C_{40}H_{56}O_2$) on the anhydrous basis.

IDENTIFICATION

• **A. SPECTROSCOPIC IDENTIFICATION TESTS (197), [Ultraviolet-Visible Spectroscopy: 197U](#)**

Wavelength range: 300–700 nm

Sample solution: Prepare as directed for the *Sample solution* in the test for *Content of Total Carotenoids*.

Acceptance criteria: It meets the requirements in the chapter. The absorbance ratio A_{446}/A_{474} is 1.09–1.14.

• **B.** The retention time for the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Lutein*.

COMPOSITION

• **CONTENT OF TOTAL CAROTENOIDS**

[NOTE—Use low-actinic glassware.]

Diluent: Hexanes, acetone, toluene, and dehydrated alcohol (10:7:7:6)

Sample stock solution: 0.3 mg/mL of Lutein in *Diluent*

Sample solution: 3.0 µg/mL of Lutein in dehydrated alcohol from dilution of *Sample stock solution*

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: UV-Vis

Analytical wavelength: 446 nm

Blank: Dehydrated alcohol

Analysis

Sample: *Sample solution*

Calculate the percentage of total carotenoids (*T*) as lutein ($C_{40}H_{56}O_2$) in the portion of Lutein taken:

$$\text{Result} = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = coefficient of extinction ($E^{1\%}$) of lutein in alcohol ($100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$), 2550

C = concentration of lutein in the *Sample solution* (g/mL)

Acceptance criteria: NLT 80.0%

• **CONTENT OF LUTEIN**

Mobile phase: Hexane and ethyl acetate (3:1)

Standard solution: 150 µg/mL of [USP Lutein RS](#) in *Mobile phase*

Sample solution: Transfer 1 mL of the *Sample stock solution* from the test for *Content of Total Carotenoids*, and evaporate under a stream of nitrogen to dryness. Add 1 mL of *Mobile phase*, and sonicate to dissolve.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV-Vis at 446 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Flow rate: 1.5 mL/min

Injection size: 10 μL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for lutein and zeaxanthin are about 1.0 and 1.05, respectively.]

Suitability requirements

Resolution: NLT 1.0 between lutein and zeaxanthin

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of the lutein peak as the total detected area in the portion of Lutein taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of lutein

r_T = sum of the responses of all the peaks

Acceptance criteria: NLT 85%

Calculate the percentage of lutein in the portion of Lutein taken:

$$\text{Result} = (r_U/r_T) \times T$$

r_U = individual peak response

r_T = sum of the responses of all the peaks

T = percentage of total carotenoids as determined in the test for *Content of Total Carotenoids*

Acceptance criteria: NLT 74.0% of lutein on the anhydrous basis

• ZEAXANTHIN AND OTHER RELATED COMPOUNDS

[NOTE—Use low-actinic glassware.]

Mobile phase, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in *Content of Lutein*.

Analysis

Sample: *Sample solution*

Calculate the percentage of zeaxanthin as the total detected area in the portion of Lutein taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of zeaxanthin

r_T = sum of the responses of all the peaks

Acceptance criteria: NMT 9.0%

Calculate the percentage of zeaxanthin in the portion of Lutein taken:

$$\text{Result} = (r_U/r_T) \times T$$

r_U = peak response of zeaxanthin

r_T = sum of the responses of all the peaks

T = percentage of total carotenoids as determined in the test for *Content of Total Carotenoids*

Calculate the percentage of other related compounds in the portion of Lutein taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = individual peak response of any other peak in the chromatogram (excluding zeaxanthin and lutein)

r_T = sum of the responses of all the peaks

Acceptance criteria: NMT 8.5% of zeaxanthin; NMT 1.0% of any other single related compound on the anhydrous basis