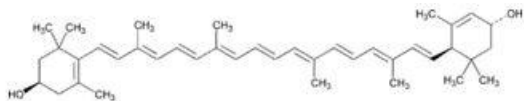


Lutein

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



β - ϵ -carotene-3,3'-diol (3R, 3'R, 6'R) [127-40-2].

Lutein is the purified fraction obtained from saponification of the oleoresin of *Tagetes erecta* L. It contains not less than 80.0 percent of total carotenoids calculated as lutein ($C_{40}H_{56}O_2$). It contains not less than 74.0 percent of lutein and not more than 8.5 percent of zeaxanthin, both calculated as ($C_{40}H_{56}O_2$) on the anhydrous basis.

Packaging and storage— Preserve in tightly sealed, light- and oxygen-resistant containers. Store in a cool place.

Identification—

A: [Ultraviolet Absorption](#) [197U](#) —

Spectral range: 300 to 700 nm.

Solution— Prepare as directed for the Test solution in the test for Content of total carotenoids.

Ratio: A446 / A474, between 1.09 and 1.14.

B: The retention time for the major peak in the chromatogram of the Test solution corresponds to that in the chromatogram of the Standard solution, as obtained in the test for Content of lutein.

[Water, Method I](#) [921](#) : not more than 1.0%.

[Residue on ignition](#) [281](#) : not more than 2.0%.

[Lead](#) [251](#) : not more than 1 μ g per g.

[Heavy metals, Method II](#) [231](#) : not more than 5 μ g per g.

Zeaxanthin and other related compounds— [note—Use low-actinic glassware.]

Solvent, Mobile phase, Standard solution, Test solution, and Chromatographic system— Proceed as directed under Content of lutein.

Procedure— Inject a volume (about 10 μ L) of the Test solution into the chromatograph, record the chromatogram, and measure the peak responses. The peak area of zeaxanthin is not more than

9.0% of the total detected area of peaks in the chromatogram of the Test solution. Calculate the percentage of zeaxanthin in the portion of Lutein taken by the formula:

$$T(r_i / r_s)$$

in which T is the content, in percentage, of total carotenoids as determined in the test for Content of total carotenoids; r_i is the individual peak response of zeaxanthin; and r_s is the sum of the responses of all the peaks: not more than 8.5% of zeaxanthin is found. Calculate the percentage of other related compounds in the portion of Lutein taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the individual peak response of any other peak in the chromatogram (excluding zeaxanthin and lutein); and r_s is the sum of the responses of all the peaks: not more than 1.0% of any other single related compound is found.

Content of lutein—

Solvent: a mixture of hexanes, acetone, toluene, and dehydrated alcohol (10:7:7:6).

Mobile phase— Prepare a filtered and degassed mixture of hexane and ethyl acetate (75:25).

Make adjustments if necessary.

Standard solution— Dissolve a suitable quantity of USP Lutein RS in Mobile phase to obtain a solution containing about 150 µg per mL.

Test solution— Transfer about 1 mL of Test 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— The liquid chromatograph is equipped with a 446-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L3. The flow rate is about 1.5 mL per minute. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 1.05 for zeaxanthin and 1.0 for lutein; the resolution, R, between lutein and zeaxanthin is not less than 1.0; the tailing factor is not more than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Inject a volume (about 10 µL) of the Test solution into the chromatograph, record the chromatogram, and measure the peak area responses. The peak area of lutein is not less than 85.0% of the total detected area of peaks in the chromatogram. Calculate the percentage of Lutein taken by the formula:

$$T(r_i / r_s)$$

in which T is the content, in percentage, of total carotenoids as determined in the test for Content of total carotenoids; r_i is the individual peak response of lutein in the Test solution; and r_s is the sum of the responses of all the peaks: not less than 74.0% of lutein is found.

Content of total carotenoids— [note—Use low-actinic glassware.]

Solvent: a mixture of hexanes, acetone, toluene, and dehydrated alcohol (10:7:7:6).

Test stock solution— Transfer about 30 mg of Lutein to a 100-mL volumetric flask, and dissolve in and dilute with Solvent to volume.

Test solution— Quantitatively dilute the Test stock solution (1 in 100) with dehydrated alcohol to obtain a solution having a final concentration of about 3.0 μg per mL.

Procedure— Determine the absorbance of the Test solution at the wavelength of maximum absorbance at about 446 nm, with a suitable spectrophotometer, using dehydrated alcohol as a blank. Calculate the percentage of total carotenoids as lutein ($\text{C}_{40}\text{H}_{56}\text{O}_2$) by the formula:

$$10,000A / 2550W$$

in which A is the absorbance of the Test solution; W is the weight, in g, of Lutein taken to prepare the Test stock solution; and 2550 is the absorptivity of the lutein in alcohol.