

Lutein Preparation

Lutein Preparation is a combination of Lutein with one or more inert substances. It may be in a solid or a liquid form. It contains not less than 95.0 percent and not more than 130.0 percent of the labeled amount of lutein, calculated as C₄₀H₅₆O₂ on the anhydrous basis. The lutein content of total carotenoids is not less than 85.0 percent, and the zeaxanthin content is not more than 9.0 percent.

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

Labeling— The label states that this article is not intended for direct administration to humans or to animals.

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 of 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 10.0%.

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

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

[Heavy metals, Method II](#) [231](#) : not more than 10 µg per g.

Zeaxanthin and other related compounds—

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

Procedure— Inject a volume (about 10 µL) of the Test solution into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of zeaxanthin relative to total carotenoids in the Preparation taken by the formula:

$$100(r_i / r_s)$$

in which 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 9.0% of zeaxanthin is found; not more than 1.0% of any other single related compound is found; and the total related compounds (including zeaxanthin) found are not more than 15.0%.

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 1.0 mL of Test stock solution 1, or 1.0 mL of Test stock solution 2, or 2.0 mL of Test stock solution 3 from the test for Content of total carotenoids into a suitable vial.

Evaporate the solvent to dryness under a stream of nitrogen. Add about 1.0 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 \times 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 responses. Calculate the percentage of lutein relative to total carotenoids in the Preparation taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the individual peak response of lutein, and r_s is the sum of the responses of all the peaks: not less than 85.0% of lutein is found. Calculate the amount of lutein in the Preparation 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.

Content of total carotenoids—

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

Test stock solution 1 (for solid lutein preparations labeled as containing gelatin)— Transfer the amount of Preparation, equivalent to about 3.5 mg of lutein, to a 50-mL centrifuge tube. Add about 15 mL of warm water, 60 units of bacterial alkaline protease preparation, and 1 mg of bromelain. Cap and sonicate for 20 minutes with occasional swirling. Cool to room temperature, and add 20.0 mL of methylene chloride. Shake for 1 minute, and centrifuge for 5 minutes at 2000 rpm. Remove the upper aqueous phase, and add 2 to 3 g of anhydrous sodium sulfate to the remaining red layer.

Test stock solution 2 (for other solid lutein preparations)— Transfer the amount of Preparation, equivalent to about 1.5 mg of lutein, to a 50-mL centrifuge tube. Add about 15 mL of warm water, cap, and sonicate for 30 minutes with occasional swirling. Cool to room temperature, and add 30.0 mL of ethyl acetate and 2 to 3 g of sodium chloride. Shake for 1 minute, and centrifuge for 5 minutes at 2000 rpm. The upper orange-red layer is Test stock solution 2.

Test stock solution 3 (for liquid lutein suspensions in oil)— Transfer an accurately weighed amount of Preparation, equivalent to about 20 mg of lutein, to a 100-mL volumetric flask, and dilute with Solvent to volume. Add a magnetic bar, and stir for 30 minutes.

Test solution— Transfer 1.0 mL of Test stock solution 1, or 1.0 mL of Test stock solution 2, or 1.0 mL of Test stock solution 3 into a 100-mL volumetric flask, and dilute with dehydrated alcohol to volume.

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 ($C_{40}H_{56}O_2$) in the Preparation by the formula:

$$VDA / 2550W$$

in which V is the volume of organic solvent (30.0 mL for Test stock solution 1, 100.0 mL for Test stock solution 2, and 100.0 mL for Test stock solution 3) used in preparing the Test stock solution;

D is the dilution factor used in preparing the Test solution; A is the absorbance of the Test solution; W is the weight, in g, of Preparation taken to prepare the appropriate Test stock solution; and 2550 is the absorptivity of the lutein in alcohol.