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 mL · g^{-1} · 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

IMPURITIES

- **RESIDUE ON IGNITION ⟨281⟩:** NMT 2.0%
- **LEAD ⟨251⟩:** NMT 1 ppm
- **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 5 ppm

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* **⟨921⟩:** NMT 1.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetically sealed, light- and oxygen-resistant containers. Store in a cool place.
- **USP REFERENCE STANDARDS ⟨11⟩**
USP Lutein RS

Lutein Preparation

DEFINITION
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 NLT 95.0% and NMT 130.0% of the labeled amount of lutein, calculated as $C_{40}H_{56}O_2$ on the anhydrous basis. It contains NLT 85.0% of lutein and NMT 9.0% of zeaxanthin of the total carotenoid content.

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION ⟨197U⟩**
Analytical wavelength: 300–700 nm
Sample solution: Prepare as directed for the *Sample solution* in the test for *Content of Total Carotenoids*.
Ratio: A_{446}/A_{474}, 1.09–1.14
- **B.** The retention time of 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**

Diluent: Hexanes, acetone, toluene, and dehydrated alcohol (10:7:7:6)
Sample stock solution A (for solid lutein preparations labeled as containing gelatin)**:** Transfer an amount of Preparation, equivalent to 3.5 mg of lutein, to a 50-mL centrifuge tube. Add 15 mL of warm water, 60 units of bacterial alkaline protease preparation, and 1 mg of bromelain. Cap and sonicate for 20 min with occasional swirling. Cool to room temperature, and add 20.0 mL of methylene chloride. Shake for 1 min, and centrifuge for 5 min at 2000 rpm. Remove the upper aqueous phase, and add 2–3 g of anhydrous sodium sulfate to the remaining red layer.

Sample stock solution B (for other solid lutein preparations)**:** Transfer an amount of Preparation, equivalent to 1.5 mg of lutein, to a 50-mL centrifuge tube. Add 15 mL of warm water, cap, and sonicate for 30 min with occasional swirling. Cool to room temperature, and add 30.0 mL of ethyl acetate and 2–3 g of sodium chloride. Shake for 1 min, and centrifuge for 5 min at 2000 rpm. Use the upper orange-red layer.

Sample stock solution C (for liquid lutein suspensions in oil)**:** Transfer a weighed amount of Preparation equivalent to 20 mg of lutein to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Add a magnetic bar, and stir for 30 min.

Sample solution: Transfer 1.0 mL of *Sample stock solution A*, or 1.0 mL of *Sample stock solution B*, or 1.0 mL of *Sample stock solution C* into a 100-mL volumetric flask, and dilute with dehydrated alcohol to volume.

Spectrometric conditions

(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)

Analytical wavelength: 446 nm

Cell path: 1 cm

Blank: Dehydrated alcohol

Analysis

Sample: *Sample solution*

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

$$\text{Result} = (A \times V \times D \times 100)/(F \times W)$$

A = absorbance of the *Sample solution*

F = absorptivity of the lutein in alcohol, 255.0 (mL/mg · cm)

V = volume of organic solvent (20.0 mL for *Sample stock solution A*, 30.0 mL for *Sample stock solution B*, and 100.0 mL for *Sample stock solution C*) used in preparing the *Sample stock solutions*

D = dilution factor used to prepare the *Sample solution* from *Sample stock solutions*

W = weight of Preparation taken to prepare the *Sample stock solutions* (mg)

- **CONTENT OF LUTEIN**

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

Mobile phase: Hexane and ethyl acetate (75:25)

Standard solution: 150 µg/mL of USP Lutein RS in *Mobile phase*

Sample solution: Transfer 1.0 mL of *Sample stock solution A*, or 1.0 mL of *Sample stock solution B*, or 2.0 mL of *Sample stock solution C* from the test for *Content of Total Carotenoids* into a suitable vial. Evaporate the solvent to dryness under a stream of nitrogen. Add 1.0 mL of *Mobile phase*, and sonicate to dissolve.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 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

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

Calculate the percentage of lutein relative to total carotenoids in the Preparation taken:

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

r_U = individual peak response of lutein

r_T = sum of the responses of all the peaks

Calculate the percentage of lutein in the Preparation taken:

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

r_U = individual peak response of lutein in the *Sample solution*

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 85.0% of lutein in the total carotenoid content, and the Preparation contains 95.0%–130.0% of the labeled amount of lutein, calculated as $C_{40}H_{56}O_2$, on the anhydrous basis.

- **ZEAXANTHIN AND OTHER RELATED COMPOUNDS**

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

Analysis

Sample: *Sample solution*

Injection size: 10 µL

Calculate the percentage of zeaxanthin relative to total carotenoids in the Preparation taken:

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

r_U = individual peak response of zeaxanthin

r_T = sum of the responses of all the peaks

Acceptance criteria

Zeaxanthin: NMT 9.0%

Any other single related compound: NMT 1.0%

Total related compounds (including zeaxanthin)**:** NMT 15.0%

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 2.0%
- **LEAD** ⟨**251**⟩**:** NMT 1 ppm
- **HEAVY METALS** ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS

- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 10.0%

ADDITIONAL REQUIREMENTS

- **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.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Lutein RS

Lycopene

$C_{40}H_{56}$ 536.88

[502-65-8].

DEFINITION

Lycopene is a mixture of geometrical isomers of lycopene. It contains NLT 96.0% and NMT 101.0% of lycopene ($C_{40}H_{56}$), calculated on the dried basis.

IDENTIFICATION

- **A. ULTRAVIOLET-VISIBLE ABSORPTION** ⟨**197U**⟩

Wavelength range: 300–700 nm

Test solution: Prepare as directed for the *Sample solution* in the test for *Content of Lycopene*.

Acceptance criteria: Meets the requirements in the chapter. The ratio of A_{476}/A_{508} is 1.10–1.14.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as