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meso-Zeaxanthin Preparation

DEFINITION

meso-Zeaxanthin Preparation is a combination of *meso*-Zeaxanthin 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 total carotenoids, calculated as zeaxanthin ($C_{40}H_{56}O_2$) on the anhydrous basis. It contains NLT 85.0% of zeaxanthin and NMT 9.0% of lutein of the total carotenoid content.

IDENTIFICATION

• A.

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

Analysis: Record the UV-Vis spectrum from 300 to 600 nm.

Acceptance criteria: The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 453 nm, and another maximum at about 480 nm.

• 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 Zeaxanthin*.

Change to read:

• C. The retention time of the major peak of the *Sample solution* corresponds to that of (3*R*,3'*S*)-zeaxanthin from the *Standard solution*, as obtained in the test for *Stereoisomeric Composition*.

COMPOSITION

• CONTENT OF TOTAL CAROTENOIDS

Sample stock solution A (for solid Preparations): Transfer an amount of Preparation equivalent to 7.5 mg of zeaxanthin to a 100-mL low-actinic volumetric flask, add 5 mL of water, and sonicate at 60° for 5 min. Add 50 mL of alcohol, and dilute with methylene chloride to volume. Shake thoroughly to obtain a fine dispersion, transfer 10 mL of this dispersion to a centrifuge tube, and centrifuge. Discard the foremost 2 mL of the supernatant. Use the clear solution.

Sample solution A: Transfer 1.0 mL of *Sample stock solution A* to a 50-mL low-actinic flask, and dilute with a mixture of cyclohexane and alcohol (9:1) to volume.

Sample stock solution B (for liquid Preparations): Transfer an amount of Preparation equivalent to 7.5 mg of *meso*-zeaxanthin to a 100-mL low-actinic volumetric flask, add 20 mL of chloroform, and sonicate for 5 min. Cool the solution to room temperature, and dilute with cyclohexane to volume. Shake thoroughly to obtain a fine dispersion, transfer 10 mL of this dispersion to a centrifuge tube, and centrifuge.

Sample solution B: Transfer 1.0 mL of *Sample stock solution B* to a 50-mL low-actinic flask, and dilute with cyclohexane to volume.

Instrumental conditions

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

Analytical wavelength: 453 nm

Cell: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

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

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

A = absorbance of the *Sample solution*

C = concentration of Preparation in *Sample solution A* or *Sample solution B* (g/mL)

F = coefficient of extinction ($E^{1\%}$) of zeaxanthin in cyclohexane ($100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$), 2540

Calculate the percentage of the labeled amount of total carotenoids:

$$\text{Result} = (T/L) \times 100$$

T = percentage of total carotenoids found as calculated above

L = labeled amount of total carotenoids (%)

Acceptance criteria: 95.0%–130.0% of the labeled amount of total carotenoids as zeaxanthin ($C_{40}H_{56}O_2$) on the anhydrous basis

• CONTENT OF ZEAXANTHIN

[NOTE—Use low-actinic glassware.]

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

Standard solution: 75 µg/mL of [USP meso-Zeaxanthin RS](#) in Mobile phase

Sample solution: Proceed as directed in *Sample stock solution A* or *Sample stock solution B* in the test for *Content of Total Carotenoids*. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 453 nm

Column: 4.6-mm × 25-cm; 3-µm packing L3

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for zeaxanthin and lutein are about 1.0 and 0.95, respectively.]

Suitability requirements

Resolution: NLT 1.0 between zeaxanthin and lutein

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Sample: *Sample solution*

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

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

r_U = individual peak response of zeaxanthin

r_T = sum of all the peak responses

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

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

r_U = individual peak response of *meso*-zeaxanthin in the *Sample solution*

r_T = sum of all the peak responses

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

Acceptance criteria: NLT 85.0% of zeaxanthin in the total carotenoid content

• LUTEIN AND OTHER RELATED COMPOUNDS

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

Injection volume: 10 µL

Analysis

Sample: *Sample solution*

Calculate the percentage of lutein and other related compounds relative to total carotenoids in the portion of Preparation taken:

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

r_U = peak response of lutein or other individual related compounds

r_T = sum of all the peak areas