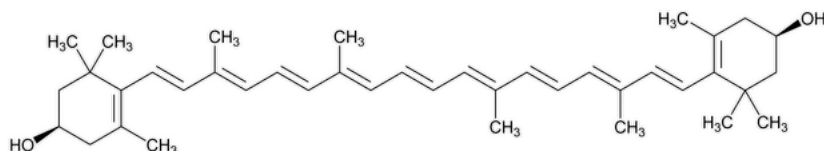


Status: Currently Official on 17-Feb-2025
 Official Date: Official as of 01-Feb-2023
 Document Type: Dietary Supplement Monographs
 DocId: GUID-FEB44754-E26C-4D56-B2C5-D5376511E759_3_en-US
 DOI: https://doi.org/10.31003/USPNF_M5756_03_01
 DOI Ref: hmh1c

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Aztec Marigold Zeaxanthin Extract



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

(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene-3-ol];

3*R*,3'*R*-β,β-Carotene-3,3'-diol CAS RN®: 144-68-3.

DEFINITION

Aztec Marigold Zeaxanthin Extract is a purified extract, derived from the flowers of *Tagetes erecta* L., grown from seeds of cultivars of Scarletade rich in zeaxanthin. The extract contains NLT 36.0% of total carotenoids calculated as zeaxanthin ($C_{40}H_{56}O_2$), NLT 30.0% of all-*trans*-zeaxanthin, and NMT 8.0% of lutein, all calculated on the dried basis.

IDENTIFICATION

• A.

Sample solution: Use the *Sample solution* from *Procedure 1: Content of Total Carotenoids*.

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

Acceptance criteria: The *Sample solution* shows a shoulder at about 428 nm, an absorption maximum at about 452 nm, and another maximum at about 479 nm.

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of zeaxanthin from the *Standard solution*, as obtained in *Procedure 2: Content of Zeaxanthin*. The *Sample solution* also exhibits a minor peak eluting before zeaxanthin corresponding to lutein. Other peaks of lower intensity than lutein might appear after zeaxanthin toward the end of the chromatographic run.

Change to read:

• **C.** The retention time of the major peak of the *Sample solution* corresponds to that of (3*R*,3'*R*)-zeaxanthin from the *Standard solution*, as obtained in *Procedure 4: Stereoisomeric Composition*. The *Sample solution* also exhibits a minor peak eluting before (3*R*,3'*R*)-zeaxanthin corresponding to (3*R*,3'*S*)-zeaxanthin, and another minor peak eluting after (3*R*,3'*R*)-zeaxanthin corresponding to (3*R*,3'*R*,6'*R*)-lutein.

COMPOSITION

• PROCEDURE 1: CONTENT OF TOTAL CAROTENOIDS

[NOTE—Use low-actinic glassware.]

Sample stock solution: Use the *Sample stock solution* from *Procedure 2: Content of Zeaxanthin*.

Sample solution: Transfer 2.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, dilute with [ethanol](#) to volume, and mix well.

Instrumental conditions

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

Analytical wavelength: 450 nm

Cell path: 1 cm

Blank: [Ethanol](#)

Analysis

Sample: *Sample solution* [NOTE—The absorbance should be between 0.2 and 1.0 absorbance units (AU). If not, adjust the dilution.]

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

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

A = absorbance of the *Sample solution*

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

F = coefficient of extinction ($E^{1\%}$) of zeaxanthin in [ethanol](#) ($100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$), 2480

Acceptance criteria: NLT 36.0% of total carotenoids (*T*) as zeaxanthin ($C_{40}H_{56}O_2$) on the dried basis

• **PROCEDURE 2: CONTENT OF ZEAXANTHIN**

[NOTE—Use low-actinic glassware.]

Solution A: [tert-Butyl methyl ether](#)

Solution B: [Water](#)

Solution C: [Methanol](#)

Diluent: [Hexane](#), [ethanol](#), [acetone](#), and [toluene](#) (10:6:7:7)

Mobile phase: Gradient elution. See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0.0	5	7	88
15	15	7	78
30	45	7	48
60	75	6.5	18.5
66	75	6	19
76	5	7	88
86	5	7	88

Standard stock solution: Accurately weigh 20 mg of [USP Aztec Marigold Zeaxanthin Extract RS](#) and transfer to a 100-mL volumetric flask, add 75 mL of *Diluent* to suspend the sample, and sonicate for 5 min. Dilute with *Diluent* to volume, and mix well. Allow the insolubles to settle for at least 10 min. Pass the supernatant through a membrane filter of 0.45-µm pore size.

Standard solution: Transfer 0.5 mL of the *Standard stock solution* to an 8-mL vial, and evaporate to dryness with the aid of a nitrogen stream. Dissolve the residue in 4.0 mL of a mixture of [tert-butyl methyl ether](#) and [methanol](#) (5:95).

Sample stock solution: Accurately weigh 20 mg of Extract and transfer to a 100-mL volumetric flask. Add 75 mL of *Diluent* to suspend the sample, and sonicate for 5 min. [CAUTION—Sample may splatter and stick to the side of the flask. Carefully wash down the product into the flask using *Diluent*.] Dilute with *Diluent* to volume, and mix well. Allow the insolubles to settle for at least 10 min. Pass the supernatant through a membrane filter of 0.45-µm pore size.

Sample solution: Transfer 0.5 mL of the *Sample stock solution* to an 8-mL vial, and evaporate to dryness with the aid of a nitrogen stream. Dissolve the residue in 4.0 mL of a mixture of [tert-butyl methyl ether](#) and [methanol](#) (5:95).

Chromatographic system

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

Mode: HPLC

Detector: 450 nm

Column: 2.0-mm × 15-cm; 3-µm packing [L62](#)

Flow rate: 0.4 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between zeaxanthin and lutein

Tailing factor: NMT 2.0 for the zeaxanthin peak

Relative standard deviation: NMT 2.0% for the zeaxanthin peak

Chromatogram similarity: The chromatogram of the *Standard solution* is similar to the reference chromatogram provided with the lot of [USP Aztec Marigold Zeaxanthin Extract RS](#) being used.

Analysis

Sample: *Sample solution*

Calculate the percentage of all-*trans*-zeaxanthin (C₄₀H₅₆O₂) in the portion of the sample taken:

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

r_U = peak area of all-*trans*-zeaxanthin from the *Sample solution*

r_T = sum of all the peak areas from the *Sample solution*