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Astaxanthin Esters

Astaxanthin esters;
Astaxanthin fatty acid esters;
Fatty acid esters of (3S,3'S)-3,3'-dihydroxy- β,β -carotene-4,4'-dione.

DEFINITION

Astaxanthin Esters is obtained by extraction with either supercritical carbon dioxide or acetone from cultures of *Haematococcus pluvialis*. It consists mainly of 3S,3'S stereoisomers of astaxanthin in the monoester, diester, and free forms. The monoester form is the most abundant, followed by the diester form. The free form is a minor component. Suitable antioxidants may be added. It contains NLT 5% of total astaxanthin, calculated as free astaxanthin on the anhydrous basis.

IDENTIFICATION

Delete the following:

▲ A. THIN-LAYER CHROMATOGRAPHY ▲ (USP 1-MAY-2024)

Add the following:

▲ A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).

Standard solution: 10 mg/mL of [USP Astaxanthin Esters from *Haematococcus pluvialis* RS](#) in [acetone](#)

Sample solution: 10 mg/mL of Astaxanthin Esters in [acetone](#)

Chromatographic system

(See standard parameters as defined in [HPTLC for Articles of Botanical Origin \(203\)](#), [Table 1](#).)

Application volume: 2 μ L

Developing solvent system: [Cyclohexane](#) and [ethyl acetate](#) (75:25)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry the plate at room temperature for 5 min. Examine the plate under white light.

System suitability: The *Standard solution* exhibits an orange band due to astaxanthin diesters in the upper-third section, as well as four broad orange bands due to astaxanthin monoesters in the middle-third section, which form two pairs of increasing intensity with the zone at the lowest R_f being the most intense.

Acceptance criteria: The *Sample solution* exhibits five bands corresponding in R_f , color, and intensity to those observed in the *Standard solution*. One or two very faint orange bands due to free astaxanthin may be observed in the lower-third section. ▲ (USP 1-May-2024)

B. HPLC

Analysis: Proceed as directed in the test for *Content of Total Astaxanthin*.

Acceptance criteria: The retention times for the three major peaks of 13-*cis*-astaxanthin, all-*trans*-astaxanthin, and 9-*cis*-astaxanthin in the *Sample solution* correspond to those in the *Standard solution*. All-*trans*-astaxanthin is the major constituent, followed by 13-*cis*-astaxanthin and 9-*cis*-astaxanthin.

ASSAY

Change to read:

• CONTENT OF TOTAL ASTAXANTHIN

[NOTE—Astaxanthin determined by this method is total astaxanthin, including the free astaxanthin, the monoester, and the diester.]

Buffer solution: Dissolve 6.06 g of tris(hydroxymethyl)aminomethane in 750 mL of [water](#), adjust with 1 N [hydrochloric acid](#) to a pH of 7.0, and dilute with [water](#) to 1000 mL.

Cholesterol esterase solution: 4 units/mL of cholesterol esterase¹ in *Buffer solution*. Prepare fresh daily.

Solution A: [Methanol](#)

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

Solution C: [Phosphoric acid](#), 1% aqueous

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

Table 1

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	81	15	4
15	66	30	4
23	16	80	4
27	16	80	4
27.1	81	15	4
35	81	15	4

Internal standard solution: 37.5 µg/mL of [USP Apocarotenal RS](#) in [acetone](#)

Standard stock solution: Transfer 30 mg of [USP Astaxanthin Esters from Haematococcus pluvialis RS](#) to a 100-mL volumetric flask. Dissolve in 30 mL of [acetone](#), shake by mechanical means, and dilute with [acetone](#) to volume.

Standard solution: Combine 2.0 mL of the *Standard stock solution* and 1.0 mL of the *Internal standard solution* in a glass centrifuge tube. Add 3.0 mL of *Cholesterol esterase solution* to the tube, and mix gently by inversion. Place the tube in a block heater set to 37°, and allow the reaction to continue for 45 min, gently and slowly inverting the tube every 10 min. After 45 min, add 1 g of ▲[sodium sulfate decahydrate](#)▲ (USP 1-May-2024) and 2 mL of [petroleum ether](#) to the tube. Mix on a vortex mixer for 30 s, and then centrifuge at 3000 rpm for 3 min. Carefully transfer the petroleum ether layer to a 10-mL glass centrifuge tube containing 1 g of [anhydrous sodium sulfate](#). Be careful to avoid pipetting the intermediate emulsive layer. Evaporate the petroleum ether layer using a vacuum or a stream of inert gas at room temperature. Add 3 mL of [acetone](#), sonicate, and filter the mixture.

Sample stock solution: Warm a quantity of the sample in a water bath at 50°–60° for 30 min. Shake the sample well at 10-min intervals. After 30 min, transfer 30 mg of the sample to a 100-mL volumetric flask. Dissolve in 30 mL of [acetone](#), shake by mechanical means, and dilute with [acetone](#) to volume.

Sample solution: Combine 2.0 mL of the *Sample stock solution* and 1.0 mL of the *Internal standard solution* in a glass centrifuge tube. Add 3.0 mL of *Cholesterol esterase solution* to the tube, and mix gently by inversion. Place the tube in a block heater set to 37°, and allow the reaction to continue for 45 min, gently and slowly inverting the tube every 10 min. After 45 min, add 1 g of ▲[sodium sulfate decahydrate](#)▲ (USP 1-May-2024) and 2 mL of [petroleum ether](#) to the tube. Mix on a vortex mixer for 30 s, and then centrifuge at 3000 rpm for 3 min. Carefully transfer the petroleum ether layer to a 10-mL glass centrifuge tube containing 1 g of [anhydrous sodium sulfate](#). Be careful to avoid pipetting the intermediate emulsive layer. Evaporate the petroleum ether layer using a vacuum or a stream of inert gas at room temperature. Add 3 mL of [acetone](#), sonicate, and filter the mixture.

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

Mode: LC
Detector: UV 474 nm
Column: 4.6-mm × 25-cm; 5-µm packing [L62](#)
Flow rate: 1.0 mL/min
Injection volume: 20 µL

System suitability
Sample: *Standard solution*

[NOTE—See [Table 2](#) for approximate relative retention times ▲ and relative response factors.▲ (USP 1-May-2024)]

Table 2

Name	▲Approximate▲ (USP 1-May-2024) Relative Retention Time	Relative Response Factor
13- <i>cis</i> -Astaxanthin	0.9	1.3
all- <i>trans</i> -Astaxanthin	1.0	1.0
9- <i>cis</i> -Astaxanthin	1.4	1.1
Apocarotenal (internal standard)	1.7	

Suitability requirements