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Astaxanthin Esters Capsules

Change to read:

DEFINITION

Astaxanthin Esters Capsules contain Astaxanthin Esters. Capsules contain NLT 90.0% and NMT 125.0% of the labeled amount of total astaxanthin, calculated as free astaxanthin. ▲Capsules may contain other labeled added substances that are generally recognized as safe, in amounts that are unobjectionable. ▲ (USP 1-May-2024)

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: Transfer NLT 5 Capsules to a small beaker and cut open with tweezers and blade. Add 30 mL of acetone and sonicate for 10 min. Remove the Capsules, transfer the contents to a volumetric flask, and fill with acetone to volume to obtain a concentration of 10 mg/mL of the labeled amount of astaxanthin esters.

Chromatographic system

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

Application volume: 2 µL

Developing solvent system 1: [Toluene](#)

Developing solvent system 2: [Cyclohexane](#) and [ethyl acetate](#) (75:25)

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Samples* as bands and dry in air. Develop the plate with *Developing solvent system 1* in an unsaturated chamber without plate activation to a distance of 85 mm from the lower edge of the plate. Remove the plate from the chamber and dry at room temperature for 5 min.

Develop the plate with *Developing solvent system 2* in a saturated chamber with plate activation at 33% relative humidity to a distance of 70 mm from the lower edge of the plate. Remove the plate from the chamber and dry 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.

STRENGTH

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 calculated as free astaxanthin.]

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: 4.0 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 80 mL of [acetone](#), shake by mechanical means, and dilute with [acetone](#) to volume. Dilute the resulting solution with acetone to obtain a concentration of 3 µg/mL of astaxanthin.

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*, 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](#). 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 under a vacuum or a stream of inert gas at room temperature, add 3 mL of acetone, sonicate, and filter the mixture.

Sample stock solution: Weigh NLT 20 Capsules in a tared weighing bottle. With a sharp blade, or by other appropriate means, carefully open the Capsules without loss of shell material and transfer the combined Capsule contents to a suitable container. Remove any adhering substance from the emptied Capsules by washing them with several portions of [diethyl ether](#). Discard the washings and allow the empty Capsules to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsules in the original tared weighing bottle and calculate the average fill weight per Capsule. Transfer an accurately weighed portion of the combined Capsules contents, equivalent to about 3 mg of the labeled amount of astaxanthin, to a 100-mL volumetric flask. Add 70 mL of [acetone](#) to dissolve, shake by mechanical means, and dilute with [acetone](#) to volume. Dilute with [acetone](#) to obtain a concentration of 3 µg/mL of astaxanthin.

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*, and mix gently by inversion. Place the tubes 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](#). 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 under 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-Vis 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 the ▲approximate▲ (USP 1-May-2024) relative retention times ▲and relative response factors.▲ (USP 1-May-2024)]

Table 2