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Beta Carotene Capsules

DEFINITION

Change to read:

Beta Carotene Capsules contain NLT ▲90.0%▲ (USP 1-May-2019) and NMT 125.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$).

▲▲ (USP 1-May-2019)

IDENTIFICATION

• **A.**

Sample solution: Dilute the *Sample stock solution* from the test for *Content of Total Beta Carotene* with cyclohexane to a final concentration of 1–5 µg/mL of beta carotene. Pass through a membrane filter of 0.45-µm pore size.

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 455 nm, and another maximum at about 483 nm. The absorbance ratio A_{455}/A_{483} is between 1.14 and 1.18.

• **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 Total Beta Carotene*.

ASSAY

Change to read:

• **Content of Total Beta Carotene**

[Note—Use low-actinic glassware.]

Mobile phase: Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyldiisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.

Diluent: 50 mg/L of butylated hydroxytoluene in alcohol

System suitability solution: Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass through a membrane filter of 0.45-µm pore size, and use the clear filtrate.

Standard stock solution: 60 µg/mL of USP Beta Carotene RS in tetrahydrofuran. [Note—The USP Beta Carotene RS is subjected to the spectrophotometric purity test at the time of analysis; see the determination of the concentration of *Standard solution A* below.]

Standard solution A: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

Determine the concentration of *Standard solution A* according to the *Analysis* of *Standard solution B*. [Note—The concentration of *Standard solution B* equals the concentration of *Standard solution A*.]

Standard solution B: Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Analytical wavelength: ▲456 nm▲ (USP 1-May-2019)

Cell: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Standard solution B*

Calculate the concentration of total beta carotene (µg/mL) as *all-trans*-beta carotene ($C_{40}H_{56}$) in *Standard solution B*:

$$\text{Result} = (A_U/a) \times F$$

A_U = average absorbance of the three preparations of *Standard solution B*

a = absorptivity of pure *all-trans*-beta carotene in cyclohexane▲▲ (USP 1-May-2019) , 250

F = conversion factor, 1000 µg/mg

Sample stock solution: Randomly select a number of Capsules, equivalent to 10–50 mg of beta carotene, with a total weight not exceeding 5 g. For powder-containing Capsules, empty the shell, and transfer shell and contents into a 250-mL volumetric flask. For Capsules containing liquid formulations, place the Capsules directly into a 250-mL volumetric flask. Add 250 mg of butylated hydroxytoluene, 0.5 mL of alkaline protease R, and 15 mL of water. Swirl the flask gently to wet the entire contents. Sonicate the flask at 50° for 30 min, and swirl the flask every 10 min. Add 100 mL of alcohol to the warm suspension, and shake vigorously. Add 110 mL of methylene chloride, and shake vigorously again. Disperse any clumps with a homogenizer, and rinse the homogenizer probe with 15 mL of methylene chloride into the flask. Allow the solution to stand in the dark until it reaches room temperature (about 2 h), dilute with methylene chloride to volume, shake vigorously, and allow the solids to settle.

Sample solution: Dilute a volume of the *Sample stock solution* with a *Diluent*–methylene chloride mixture (1:1) so that the final concentration of beta carotene is 1–5 µg/mL. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

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

Mode: LC

Detector: UV 448 nm

Column: 4.6-mm × 25-cm; 5-µm packing L68

Column temperature: 30°

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Samples: *System suitability solution* and *Standard solution A*

[NOTE—The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1*.]

Table 1

Name	Relative Retention Time	Relative Response Factor
all-trans-Alpha carotene	0.93	▲1.05▲ (USP 1-May-2019)
all-trans-Beta carotene	1.00	1
9-*cis*-Beta carotene	1.07	1
13-*cis*-Beta carotene	1.17	1.2
15-*cis*-Beta carotene	1.21	1.4

Suitability requirements

Chromatogram similarity: The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Beta Carotene System Suitability RS being used.

Resolution: NLT ▲1.2▲ (USP 1-May-2019) between *all-trans*-beta carotene and *all-trans*-alpha carotene and between *all-trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

Tailing factor: NMT 2.0 for the *all-trans*-beta carotene peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the *all-trans*-beta carotene peak from replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution*. Measure the peak area responses.

Calculate the percentage of the labeled amount of total beta carotene in the portion of Capsules taken:

$$\text{Result} = (\Sigma r_U / r_S) \times (C_S / C_U) \times 100$$

r_U = (peak area of *all-trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) from the *Sample solution*

r_S = peak area of *all-trans*-beta carotene from *Standard solution A*

C_S = concentration of *all-trans*-beta carotene in *Standard solution A* as determined above (µg/mL)

C_U = nominal concentration of total beta carotene in the *Sample solution* (µg/mL)

Calculate the percentage of *all-trans*-beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_{all\text{-}trans}/\Sigma r_U) \times 100$$

$r_{all\text{-}trans}$ = peak area of *all-trans*-beta carotene from the *Sample solution*

r_U = (peak area of *all-trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene × 1.2) + (peak area of 15-*cis*-beta carotene × 1.4) from the *Sample solution*

Acceptance criteria: ▲90.0%▲ (USP 1-May-2019) –125.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$)

▲▲ (USP 1-May-2019)

SPECIFIC TESTS

Change to read:

• **Alpha Carotene and Other Related Compounds**

Mobile phase, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the test for *Content of Total Beta Carotene*.

Analysis

Sample: *Sample solution*

Calculate the percentage of alpha carotene and other individual related compounds relative to total beta carotene in the portion of Capsules taken:

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

r_U = peak area of alpha carotene or other individual related compounds

r_T = sum of the areas of all the peaks

Acceptance criteria

Alpha carotene: NMT 1.0%

Total related compounds (including alpha carotene): NMT ▲5%▲ (USP 1-May-2019)

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers.

Change to read:

• **Labeling:** The label states the name and content of any carriers and antioxidants added to the formulation and the content of total carotenoids as beta carotene. ▲The label states the percentages of *cis*- and *all-trans*-isomers in the total beta carotene at the time of product manufacture and release.▲ (USP 1-May-2019)

• **USP Reference Standards ⟨11⟩**

USP Beta Carotene RS

(*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].

$C_{40}H_{56}$ 536.87

USP Beta Carotene System Suitability RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
BETA CAROTENE CAPSULES	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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