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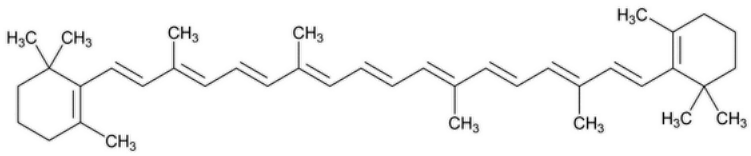
Beta Carotene



$C_{40}H_{56}$ 536.87

β,β-Carotene;

all-*trans*-β-Carotene;

(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] CAS RN®: 7235-40-7; UNII: 01YAE03M7J.

DEFINITION

Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene ($C_{40}H_{56}$). It contains NLT 95% of all-*trans*-beta carotene in the total carotenoids content.

IDENTIFICATION

• **A.**

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

Analysis: Record the UV-Vis spectrum from 300–600 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 Beta Carotene*.

COMPOSITION

• **Content of Total Carotenoids**

[Note—Use low-actinic glassware.]

Sample stock solution: 0.1 mg/mL of Beta Carotene in tetrahydrofuran

Sample solution: Transfer 3.0 mL of *Sample stock solution* to a 100-mL volumetric flask, and dilute with cyclohexane to volume.

Instrumental conditions

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

Analytical wavelength: 456 nm

Cell path: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

Calculate the percentage of total carotenoids (*T*) as beta carotene ($C_{40}H_{56}$):

$$T = A/(F \times C)$$

A = absorbance of the *Sample solution*

F = 2505, coefficient of extinction ($E^{1\%}$) of pure all-*trans*-beta carotene in cyclohexane (100 mL · g^{-1} · cm^{-1})

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

Acceptance criteria: 96.0%–101.0% of total carotenoids as beta carotene ($C_{40}H_{56}$)

• **Content of Beta Carotene**

[Note—Use low-actinic glassware.]

Mobile phase: Transfer 50 mg of butylated hydroxytoluene to 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 µg/mL 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 the suspension through a membrane filter of 0.45-µm pore size, and use the clear filtrate.

Standard solution: 10 µg/mL of USP Beta Carotene RS in tetrahydrofuran and *Diluent* (1:9). Dissolve an appropriate amount of USP Beta Carotene RS in a volumetric flask first with tetrahydrofuran, using 10% of the volume of the flask, then dilute with *Diluent* to volume.

Sample solution: Dilute the freshly prepared *Sample stock solution* as prepared in the test for *Content of Total Carotenoids* (1 in 10) with *Diluent*.

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*

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

Table 1

Analyte	Relative Retention Time	Relative Response Factor
all-*trans*-Alpha carotene	0.93	1.0
all-*trans*-Beta carotene	1.00	1.0
9-*cis*-Beta carotene	1.07	1.0
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 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene; NLT 1.2 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*

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

Analysis

Sample: *Sample solution*

Record the chromatograms, and 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 all-*trans*-beta carotene relative to total carotenoids in the sample taken:

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

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

r_T = [(peak area of all-*trans*-alpha carotene × 1.0) + (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) + (sum of peak areas of other *cis*-isomers of beta carotene)] from the *Sample solution*

Acceptance criteria: NLT 95% of all-*trans*-beta carotene in the total carotenoids content

- **Alpha Carotene and Other Related Compounds**

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

Analysis

Sample: *Sample solution*

Calculate the percentage of alpha carotene and other individual related compounds relative to total carotenoids in the portion of the *Sample* taken:

Result = $(r_U/r_T) \times 100$

r_U = (peak area of all-*trans*-alpha carotene × 1.0) or (peak area response of other individual related compounds × appropriate relative response factor, *Table 1*) in the *Sample solution*

r_T = [(peak area of all-*trans*-alpha carotene × 1.0) + (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) + (sum of peak areas of other *cis*-isomers of beta carotene)] from the *Sample solution*

Acceptance criteria

Alpha carotene: NMT 1.0%

Total related compounds (including alpha carotene)**:** NMT 5%

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.2%, 2 g of specimen being used

SPECIFIC TESTS

• **Loss on Drying ⟨731⟩**

Analysis: Dry under vacuum over phosphorus pentoxide at 40° for 4 h.

Acceptance criteria: NMT 0.2%

ADDITIONAL REQUIREMENTS

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

• **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].

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	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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