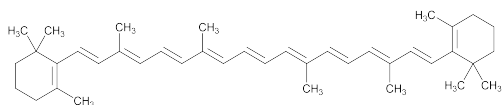


Beta Carotene



C₄₀H₅₆ 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] [7235-40-7].

DEFINITION

Change to read:

Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene (C₄₀H₅₆). It contains NLT 95% (RB 1-Dec-2014) of all-*trans*-beta carotene in the total carotenoids content.

- (RB 1-Dec-2014)

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 A₄₅₅/A₄₈₃ 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

Change to read:

• 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 *Spectrophotometry and Light-Scattering* (851).)

Analytical wavelength: 456 (RB 1-Dec-2014) nm

Cell path: 1 cm

Blank: Cyclohexane

Analysis

Sample: *Sample solution*

Calculate the percentage of total carotenoids (*T*) as beta carotene (C₄₀H₅₆):

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

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 (C₄₀H₅₆)

Change to read:

• 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*-ethyl-diisopropylamine, 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- <i>trans</i> -Alpha carotene	0.93	1.0 (RB 1-Dec-2014)
all- <i>trans</i> -Beta carotene	1.00	1.0
9- <i>cis</i> -Beta carotene	1.07	1.0
13- <i>cis</i> -Beta carotene	1.17	1.2
15- <i>cis</i> -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 (RB 1-Dec-2014) 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*

2 Beta Carotene

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 (RB 1-Dec-2014)) + (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% (RB 1-Dec-2014) of all-*trans*-beta carotene in the total carotenoids content (RB 1-Dec-2014)

Change to read:

- **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:

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

r_U = (peak area of all-*trans*-alpha carotene × 1.0 (RB 1-Dec-2014)) 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 (RB 1-Dec-2014)) + (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% (RB 1-Dec-2014)

IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%, 2 g of specimen being used

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-Dec-2015)

SPECIFIC TESTS

Delete the following:

- **MELTING RANGE OR TEMPERATURE (741):** 176°–182°, with decomposition (RB 1-Dec-2014)
- **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