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Beta Carotene Preparation

DEFINITION

Beta Carotene Preparation is a combination of beta carotene with one or more inert substances. It may be in a solid or a liquid form. It contains NLT 95.0% and NMT 130.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$) on the anhydrous basis.

IDENTIFICATION

• A.

Sample solution: Transfer 5.0 mL of *Sample stock solution A* or *Sample stock solution B* from the test for *Content of Beta Carotene* to a 100-mL volumetric flask, and dilute with [cyclohexane](#) to volume. Pass the solution through a membrane filter of 0.45- μ m 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 Beta Carotene*.

COMPOSITION

• 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 μ g/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- μ m pore size, and use the clear filtrate.

Standard stock solution: 60 μ g/mL of [USP Beta Carotene RS](#) in [tetrahydrofuran](#)

Standard solution A: Transfer 5.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of [tetrahydrofuran](#), and dilute with *Diluent* to volume. The concentration of the all-*trans*-beta carotene in this solution will be determined by the spectrophotometric procedure using *Standard solution B* as follows.

Standard solution B: Transfer 5.0 mL of the *Standard stock solution* to 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

Cell path: 1 cm

Blank: [Cyclohexane](#)

Analysis

Sample: *Standard solution B*

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

$$\text{Result} = A/F$$

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

F = absorptivity of pure all-*trans*-beta carotene in cyclohexane, 250.5

Sample stock solution A (for solid Beta Carotene Preparations): Transfer a quantity of Preparation, equivalent to 10 mg of beta carotene, to a 250-mL volumetric flask. Add 250 mg of [butylated hydroxytoluene](#), 0.5 mL of alkaline protease R, and 15 mL of [water](#). Tilt the flask gently to wet the entire contents. Sonicate the solution in an ultrasonic bath at about 50° for 30 min, and swirl at 10-min intervals. Add 100 mL of [alcohol](#) to the warm suspension, and shake vigorously. Add 135 mL of [methylene chloride](#), and shake again. Let the mixture stand in the dark until it reaches room temperature (about 2 h). Dilute with [methylene chloride](#) to volume, shake vigorously, and allow solids to settle in the dark.

Sample stock solution B (for liquid Beta Carotene suspensions in oil Preparations): Transfer a quantity of Preparation, equivalent to 20 mg of beta carotene, to a 250-mL volumetric flask. Add 250 mg of [butylated hydroxytoluene](#), 120 mL of [methylene chloride](#), and 100 mL of [alcohol](#). Shake the flask until the sample is completely dissolved or suspended. Let the mixture stand in the dark until it reaches room temperature (about 2 h). Add [methylene chloride](#) to volume, and shake again vigorously.

Sample solution: Transfer 5.0 mL of *Sample stock solution A* or *Sample stock solution B* to a 50-mL volumetric flask, and dilute with a mixture of [methylene chloride](#) and *Diluent* (1:1) to volume. 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*

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	1.0
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

Resolution: NLT 1.2 between beta carotene and alpha carotene and between beta carotene and 9-cis-beta carotene, *System suitability solution*

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

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

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.

Analysis

Samples: *Standard solution A* and *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 the labeled amount of total beta carotene in the portion of Preparation taken:

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

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) + (sum of peak areas of other cis-isomers of beta carotene)] in the *Sample solution*

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

C_S = concentration of all-trans-beta carotene in *Standard solution A* as determined by spectrometric procedure (mg/mL)

C_U = nominal concentration of Preparation in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of all-trans-beta carotene in the portion of Preparation taken:

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

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

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