Beta Carotene Preparation

Type of Posting	Revision Bulletin
Posting Date	29–Jan–2016
Official Date	01–Feb–2016
Expert Committee	Non-Botanical Dietary Supplements
Reason for Revision	Compliance

In accordance with the Rules and Procedures of the Council of Experts, the Monographs-Non-Botanical Dietary Supplements Expert Committee has revised the Beta Carotene Preparation monograph. The purpose for the revision is to remove the specification for the all-*trans*-beta carotene content of NLT 95.0% stated in the *Definition* section and in the Acceptance criteria of the test for *Content of Beta Carotene*.

Additionally, the Expert Committee requested the disclosure of the percentages of *cis*- and *trans*-isomers in the total beta carotene at the time of product manufacture and release be added to the *Labeling* section. Other revisions were also made to resolve the compliance issues.

- Change the relative response factor of all-*trans*-alpha carotene from 1.1 to 1.0 in the tests for *Content of Beta Carotene*.
- Change the resolution requirement between all-*trans*-beta carotene and 9-*cis*-beta carotene from 1.5 to 1.2 in the test for *Content of Beta Carotene*.
- Change the acceptance criteria for Total related compounds in the test for *Alpha Carotene and Other Related compounds* from NMT 5.0% to NMT 5% (no decimal figure).
- Additionally, minor editorial changes have been made to update the monograph to current *USP* style.

The Beta Carotene Preparation Revision Bulletin supersedes the currently official monograph. This Revision Bulletin will be incorporated in the *Second Supplement* to the *USP 39–NF 34.*

Should you have any questions, please contact Huy Dinh, Senior Scientific Liaison (301–816–8594 or htd@usp.org.)

Beta Carotene Preparation

DEFINITION

Change to read:

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. •● (RB 1-Feb-2016)

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-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 Beta Carotene*.

COMPOSITION

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*-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 stock solution: 60 µg/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⟩.)● (CN 1-May-2016)

  Analytical wavelength: •456 nm● (RB 1-Feb-2016)
  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● (RB 1-Feb-2016)
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• (RB 1-Feb-2016) 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• (RB 1-Feb-2016) 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• (RB 1-Feb-2016) 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*
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)

Acceptance criteria: The Preparation contains 95.0%–130.0% of the labeled amount of total beta carotene, calculated as ($C_{40}H_{56}$) on the anhydrous basis.•• (RB 1-Feb-2016)

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 Beta Carotene*.
Injection volume: 20 µL
Analysis
Sample: *Sample solution*
Calculate the percentage of alpha carotene and other individual related compounds relative to total beta carotene in the portion of Preparation taken:

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

r_U = peak area of alpha carotene or other individual related compounds from the *Sample solution*
r_T = sum of the peak areas of all the peaks from the *Sample solution*

Acceptance criteria
Alpha carotene: NMT 1.0%
Any other individual related compound: NMT 1.0%
Total related compounds (including alpha carotene)**:** NMT •5%• (RB 1-Feb-2016)

IMPURITIES
- **RESIDUE ON IGNITION ⟨281⟩:** NMT 2.0%

Delete the following:

•• **HEAVY METALS,** *Method II* **⟨231⟩:** NMT 10 ppm• (Official 1-Jan-2018)

SPECIFIC TESTS
- **WATER DETERMINATION ⟨921⟩,** *Method I***:** NMT 8.0% for solid Preparations; NMT 1.0% for liquid Preparations

ADDITIONAL REQUIREMENTS
- **PACKAGING AND STORAGE:** Preserve in tightly sealed, light- and oxygen-resistant containers. Store in a cool place.

Change to read:

- **LABELING:** The label states the name and content of any carriers and antioxidants added to the formulation, the content of total carotenoids as beta carotene, •and the percentages of *cis*- and all-*trans*-isomers in the total beta carotene at the time of product manufacture and release.• (RB 1-Feb-2016)
- **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