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. *1* (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-V is spectrum from 300 to 600 nm. Acceptance criteria: 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.

- **B.** The retention time of the major peak of the Sample solution in the ultrasonic bath at about 50 °C. Add 50 mg of butylated hydroxytoluene, 0.5 mL of alkaline protease R, and 1.5 mL of water. Tilt the flask gently to wet the entire contents. Sonicate the solution in an ultrasonic bath at about 50°C 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 A** (for solid Beta Carotene suspensions in oil 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 1.5 mL of water. Tilt the flask gently to wet the entire contents. Sonicate the solution in an ultrasonic bath at about 50°C 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.

**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 dilute with 20 mL of 2-propanol. Add 0.2 mL of N-ethylidiiisopropylamine, 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, and 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).) 1 (CN 1-May-2016)

**Analytical wavelength:** 456 nm 1 (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:

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 1.5 mL of water. Tilt the flask gently to wet the entire contents. Sonicate the solution in an ultrasonic bath at about 50°C 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>all-trans-Alpha carotene</td>
<td>0.93</td>
<td>1.0 1 (RB 1-Feb-2016)</td>
</tr>
<tr>
<td>all-trans-Beta carotene</td>
<td>1.00</td>
<td>1.0</td>
</tr>
<tr>
<td>9-cis-Beta carotene</td>
<td>1.07</td>
<td>1.0</td>
</tr>
<tr>
<td>13-cis-Beta carotene</td>
<td>1.17</td>
<td>1.2</td>
</tr>
<tr>
<td>15-cis-Beta carotene</td>
<td>1.21</td>
<td>1.4</td>
</tr>
</tbody>
</table>

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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:

\[
\text{Result} = \frac{r_0}{r_s} \times \frac{C_U}{C_s} \times 100
\]

\[r_0 = \frac{(\text{peak area of all-trans-beta carotene}) + (\text{peak area of 9-cis-beta carotene}) + (\text{peak area of 13-cis-beta carotene} \times 1.2) + (\text{peak area of 15-cis-beta carotene} \times 1.4) + (\text{sum of peak areas of other cis-isomers of beta carotene})}{\text{peak area of all-trans-beta carotene in the Sample solution}}\]

\[r_s = \frac{(\text{peak area of alpha carotene}) + (\text{sum of peak areas of other cis-isomers of alpha carotene})}{\text{nominal concentration of Preparation in the Sample solution (mg/mL)}}\]

Calculate the percentage of the labeled amount of total alpha carotene in the portion of Preparation taken:

\[
\text{Result} = \frac{r_0}{r_s} \times \frac{C_U}{C_s} \times 100
\]

\[r_0 = \frac{(\text{peak area of all-trans-beta carotene})}{\text{peak area of all-trans-beta carotene in the Sample solution}}\]

\[r_s = \frac{(\text{peak area of all-trans-beta carotene})}{\text{peak area of all-trans-beta carotene in the Standard solution A}}\]

\[C_s = \frac{(\text{concentration of all-trans-beta carotene in Standard solution A as determined by spectrometric procedure (mg/mL)})}{\text{nominal concentration of Preparation in the Sample solution (mg/mL)}}\]

Acceptance criteria: The Preparation contains 95.0%–130.0% of the labeled amount of total beta carotene, as calculated as (C_{ao}H_{18}O) on the anhydrous basis.

\* (98 1-Feb-2016)

**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:

\[
\text{Result} = \frac{r_0}{r_s} \times \left(\frac{C_U}{C_s}\right) \times 100
\]

\[r_0 = \frac{(\text{peak area of alpha carotene}) + (\text{sum of peak areas of other cis-isomers of beta carotene})}{\text{peak area of alpha carotene in the Sample solution}}\]

\[r_s = \frac{(\text{peak area of alpha carotene}) + (\text{sum of peak areas of all the peaks from the Sample solution}}}{\text{nominal concentration of Preparation in the Sample solution (mg/mL)}}\]

Acceptance criteria: Alpha carotene: NMT 1.0%
Any other individual related compound: NMT 1.0%
Total related compounds (including alpha carotene): NMT 10% (98 1-Feb-2016)

IMPURITIES
- Residue on Ignition (281): NMT 2.0%

Delete the following:
- Heavy Metals, Method II (231): NMT 10 ppm (98 1-Feb-2016)

SPECIFIC TESTS
- Water Determination (921), Method II: 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.

USP Reference Standards (11)
USP Beta Carotene RS
\((\text{all-}E)-1,1\’\’-(3,7,12,16-
\text{Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-
\text{diyl)}bis[2,6,6-trimethylcyclohexene}].
\]
USP Beta Carotene System Suitability RS