ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described under *Injections* ⟨1⟩, *Containers for Sterile Solids*. Store at controlled room temperature.
- **LABELING:** It meets the requirements for *Injections* ⟨1⟩, *Labeling*.

Change to read:

- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Azaerythromycin A RS
9-Deoxo-9a-aza-9a-homoerythromycin A.
$C_{37}H_{70}N_2O_{12}$ 734.96

USP Azithromycin RS

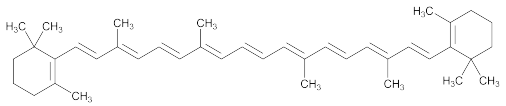
USP Azithromycin *N*-Oxide RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{38}H_{72}N_2O_{13}$ 764.98

USP *N*-Demethylazithromycin RS
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{37}H_{70}N_2O_{12}$ 734.96

USP Desosaminylazithromycin RS
■(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.
$C_{30}H_{58}N_2O_9$ 590.79■2S *(USP35)*

USP Endotoxin RS

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] [7235-40-7].

DEFINITION

Change to read:

■Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene ($C_{40}H_{56}$). It contains NLT 95.0% of all-*trans*-beta carotene in the total carotenoids content.■2S *(USP35)*

IDENTIFICATION

Change to read:

- **A.**

■**Sample solution:** Prepare as directed for 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 1.14–1.18.■2S *(USP35)*

Change to read:

- **B.** ■The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Content of Beta Carotene*.■2S *(USP35)*

ASSAY

Delete the following:

■• **PROCEDURE**

[NOTE—Perform this procedure in subdued light, using low-actinic glassware.]
Sample stock solution: Dissolve 50 mg of Beta Carotene in 10 mL of acid-free chloroform in a 100-mL volumetric flask. Dilute with cyclohexane to volume. Pipet 5 mL into a 100-mL volumetric flask, and dilute with cyclohexane.
Sample solution: *Sample stock solution* with cyclohexane (1 in 10)
Spectrometric conditions
Analytical wavelength: 455 nm
Blank: Cyclohexane
Analysis
Samples: *Sample solution* and *Blank*
Determine the absorbance of *Sample solution* using the *Blank*. Calculate the percentage of $C_{40}H_{56}$ in the portion of Beta Carotene taken:

$$\text{Result} = [(400 \times A_U)/A_S] \times 100$$

A_U = absorbance of the *Sample solution*
A_S = absorptivity of pure beta carotene, 250
Acceptance criteria: 96.0%–101.0%■2S *(USP35)*

COMPOSITION

Add the following:

■• **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* into a 100-mL volumetric flask, and dilute with cyclohexane to volume.
Instrumental conditions
(See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
Analytical wavelength: 457 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 *Sample solution* (g/mL)
Acceptance criteria: 96.0%–101.0% of total carotenoids as beta carotene ($C_{40}H_{56}$)■2S *(USP35)*

Add the following:

■• **CONTENT OF BETA CAROTENE**

[NOTE—Use low-actinic glassware.]

Mobile phase: Transfer 50 mg of butylated hydroxytoluene into 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, 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 complete 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 size: 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	1.1
all-*trans*-Beta carotene	1.00	1
9-*cis*-Beta carotene	1.07	1
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 USP Beta Carotene System Suitability RS being used.

Resolution: NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene and 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 in the chromatogram of the *Sample solution* by comparing with those in the chromatogram 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 response of all-*trans*-beta carotene from the *Sample solution*

r_T = [(peak area of all-*trans*-alpha carotene × 1.1) + (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 area of other *cis*-isomers of beta carotene)] from the *Sample solution*

Acceptance criteria: NLT 95.0% of all-*trans*-beta carotene in the total carotenoids content■2S (*USP35*)

Add the following:

■• **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*

Injection size: 20 µL

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

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

r_U = (peak area response of all-*trans*-alpha carotene × 1.1) 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 × 1.1) + (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 area of other *cis*-isomers of beta carotene)] in the *Sample solution*

Acceptance criteria

Alpha carotene: NMT 1.0%

Total related compounds (including alpha carotene)**:** NMT 5.0%■2S (*USP35*)

IMPURITIES

- **RESIDUE ON IGNITION** ⟨**281**⟩**:** NMT 0.2%, 2 g of specimen being used
- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 10 ppm

SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** ⟨**741**⟩**:** 176°–182°, with decomposition
- **LOSS ON DRYING** ⟨**731**⟩**:** Dry a sample in vacuum over phosphorus pentoxide at 40° for 4 h: it loses NMT 0.2% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

Add the following:

■• **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■2S (*USP35*)

Beta Carotene Capsules

DEFINITION

Change to read:

■Beta Carotene Capsules contain NLT 90% and NMT 125.0% of the labeled amount of total beta carotene ($C_{40}H_{56}$), of which NLT 95.0% is the all-*trans*-beta carotene isomer.■2S (*USP35*)

IDENTIFICATION

Delete the following:

■• **PROCEDURE**
Analysis: Grind a portion of the Capsule contents equivalent to 10 mg of beta carotene. Transfer to a centrifuge tube, and add 5 mL of chloroform. Shake for 1 min, and centrifuge for 3 min. Filter the supernatant layer, collecting 2 mL of the filtrate in a 25-mL conical flask. Add 5 mL of antimony trichloride TS to the filtrate.
Acceptance criteria: A transient purple or blue color forms.■2S (*USP35*)

Add the following:

■• **A**.
Sample solution: Dilute the *Sample stock solution* of the test for *Content of Total Beta Carotene* with cyclohexane to a final concentration of between 1 and 5 µg/mL of beta carotene. Pass 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.■2S (*USP35*)

Add the following:

■• **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 Total Beta Carotene*.■2S (*USP35*)

ASSAY

Delete the following:

■• **PROCEDURE**
[NOTE—Perform this analysis in subdued light, using low-actinic glassware.]
Cyclohexane: Spectrophotometric grade, or material that has been purified by being passed through a column of activated silica gel and distilled.
Iodine solution: 0.01 mg/mL of iodine in cyclohexane. [NOTE—Prepare this solution fresh daily.]
Standard solution 1: 17 mg of beta carotene, previously subjected to the *Assay* and previously dried in a vacuum over phosphorus pentoxide at 40° for 4 h. Transfer to a 1000-mL volumetric flask. Add 10 mL of water, heat at 60° for 15 min, and cool to room temperature. Add 3 g of sodium sulfate decahydrate and 2 mL of 1 N hydrochloric acid, and shake by mechanical means for 10 min. Add 200 mL of chloroform, and shake for 10 min. Add 750 mL of chloroform, shake, and dilute with chloroform to volume, disregarding the aqueous layer.
Standard solution 2: Shake *Standard solution 1* vigorously, and allow the layers to separate completely. Transfer 20 mL of the chloroform layer to a centrifuge tube, add 2 g of anhydrous sodium sulfate, shake vigorously, and allow to settle. Transfer 5.0 mL to a 50 mL volumetric flask, and add 30 mL of cyclohexane. Add 0.05 mL of *Iodine solution*, dilute with cyclohexane to volume, and allow to stand for 3 h. Transfer 20 mL of *Standard solution 2* to a centrifuge tube, and centrifuge for 2 min.
Sample solution 1: Combine the contents of NLT 20 Capsules, and grind, using a freezer mill, to a fine powder of uniform color. Transfer a quantity of the finely ground Capsule contents, equivalent to 75 mg of beta carotene, to a 1000-mL volumetric flask. Add 500 mL of water, and heat at 60° for 15 min. Cool to ambient temperature, and dilute with water to volume.
Sample solution 2: Transfer 5.0 mL of *Sample solution 1* to a glass-stoppered, 50-mL centrifuge tube. Add 3 g of sodium sulfate decahydrate, 2 mL of 1 N hydrochloric acid, and 20.0 mL of chloroform. Shake by mechanical means for 10 min, centrifuge for 5 min, and remove the aqueous layer without disturbing the chloroform layer. Add 2 g of anhydrous sodium sulfate to the chloroform layer, shake vigorously, and allow to settle.
Sample solution 3: Transfer 5.0 mL of *Sample solution 2* to a 50-mL volumetric flask, and add 30 mL of cyclohexane. Add 0.05 mL of *Iodine solution*, dilute with cyclohexane to volume, and allow to stand for 3 h. Transfer 20 mL of this solution to a centrifuge tube, and centrifuge for 2 min.
Spectrometric conditions
Analytical wavelength: 452 nm
Blank: Cyclohexane
Analysis
Samples: *Standard solution 2*, *Sample solution 3*, and *Blank*
Measure the absorbances using the *Blank*.
Calculate the percentage of $C_{40}H_{56}$ in the portion of Capsule contents taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

A_U = absorbance of *Sample solution 3*
A_S = absorbance of *Standard solution 2*
C_S = concentration of beta carotene in the *Standard solution* (µg/mL)
C_U = nominal concentration of beta carotene in *Sample solution 3* (µg/mL)
Acceptance criteria: 90.0%–125.0%■2S (*USP35*)

Add the following:

■• **CONTENT OF TOTAL BETA CAROTENE**
[NOTE—Use low-actinic glassware.]
Mobile phase: Transfer 50 mg of butylated hydroxytoluene into 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.