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Add the following:

## ^Carotenes

### DEFINITION

Carotenes consist mainly of beta carotene and alpha carotene that are obtained from palm oil, carrots, or other edible sources. They contain NLT 90% and NMT 110% of the labeled amount of total carotenes on the anhydrous basis. They contain NLT 90% and NMT 110% of the labeled amount each of beta carotene and alpha carotene on the anhydrous basis. The content of alpha carotene and beta carotene is NLT 25% and NLT 60%, respectively, of total carotenes on the anhydrous basis. Carotenes may be standardized with edible oils.

### IDENTIFICATION

#### • A.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* from the test for *Content of Total Carotenes* into 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* exhibits a shoulder at about 427 nm, an absorption maximum at about 440–457 nm, and another absorption maximum at about 470–486 nm.

• B. The chromatogram of the *Sample solution* obtained in the test for *Content of Alpha and Beta Carotenes* exhibits two main peaks due to alpha carotene and beta carotene. These two peaks correspond to those of the *System suitability solution*, with beta carotene being the most abundant, followed by alpha carotene. Additional minor peaks corresponding to 13-*cis*-beta carotene, *cis*-alpha carotene, and 9-*cis*-beta carotene may be observed.

### COMPOSITION

#### • CONTENT OF TOTAL CAROTENES

[NOTE—Use low-actinic glassware.]

**Sample stock solution:** Warm the Carotenes to 60°–65° and mix to homogenize. Transfer an amount of Carotenes, equivalent to 5 mg of total carotenes to a 50-mL volumetric flask, dissolve, and dilute with [tetrahydrofuran](#) to volume.

**Sample solution:** Transfer 3.0 mL of the *Sample stock solution* into a 100-mL volumetric flask, and dilute with [cyclohexane](#) to volume.

#### Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

**Analytical wavelength:** Use the maximum absorbance between 440 and 457 nm.

**Cell path:** 1 cm

**Blank:** [Cyclohexane](#)

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of the labeled amount of total carotenes as beta carotene in the portion of sample taken:

$$\text{Result} = A_U / (F \times C_U)$$

$A_U$  = absorbance of the *Sample solution*

$F$  = 2500, coefficient of extinction ( $E^{1\%}$ ) of pure all-*trans*-beta carotene in cyclohexane ( $100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ )

$C_U$  = nominal concentration of total carotenes in the *Sample solution* (g/mL)

**Acceptance criteria:** 90.0%–110% on the anhydrous basis

#### • CONTENT OF ALPHA AND BETA CAROTENES

[NOTE—Use low-actinic glassware.]

**Solution A:** [Water](#), methanol, and [tert-butyl methyl ether](#) (4:81:15)

**Solution B:** [Water](#), methanol, and [tert-butyl methyl ether](#) (4:15:81)

**Mobile phase:** See [Table 1](#).

Table 1

| Time<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 0             | 100               | 0                 |
| 30            | 0                 | 100               |
| 40            | 100               | 0                 |
| 50            | 100               | 0                 |

**System suitability solution:** Warm the [USP Carotenes RS](#) to 60°–65° and mix to homogenize. Transfer an amount of standard, equivalent to 5 mg of total carotenes, to a 50-mL volumetric flask, dissolve, and dilute with [tetrahydrofuran](#) to volume. Transfer 3.0 mL of the resultant solution to a 50-mL volumetric flask and dilute with [cyclohexane](#) to volume.

**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* into a 100-mL volumetric flask, and dilute with [cyclohexane](#) to volume. The concentration of 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* into a 100-mL volumetric flask, and dilute with [cyclohexane](#) to volume. Prepare in triplicate.

#### Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

**Analytical wavelength:** Use the maximum absorbance between 440 and 457 nm.

**Cell path:** 1 cm

**Blank:** [Cyclohexane](#)

#### Analysis

**Sample:** *Standard solution B*

Calculate the concentration of total beta carotene, in mg/mL, in *Standard solution B*:

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

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

*F* = absorptivity of pure beta carotene in cyclohexane, 250

**Sample stock solution:** Warm the Carotenes to 60°–65° and mix to homogenize. Transfer an amount of Carotenes, equivalent to 5 mg of total carotenes, to a 50-mL volumetric flask, dissolve, and dilute with [tetrahydrofuran](#) to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* into a 100-mL volumetric flask, and dilute with [cyclohexane](#) to volume.

#### Chromatographic system

(See [Chromatography \(621\)](#), *System Suitability*.)

**Mode:** LC

**Detector:** UV. Use the maximum absorbance between 440 and 457 nm.

**Column:** 4.6-mm × 25-cm; 5-µm packing L62

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution A*

#### Suitability requirements

**Chromatogram similarity:** The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of [USP Carotenes RS](#) being used. The approximate relative retention times of the components in the *System suitability solution* are listed in [Table 2](#).

**Table 2**

| Name                              | Relative Retention Time | Correction Factor <sup>a</sup> |
|-----------------------------------|-------------------------|--------------------------------|
| 15- <i>cis</i> -Beta carotene     | 0.88                    | 1.4                            |
| 13- <i>cis</i> -Beta carotene     | 0.91                    | 1.2                            |
| All- <i>trans</i> -alpha carotene | 0.93                    | 1.05                           |