Asiamerica Group, Inc.
85 Chestnut Ridge Road, Suite 218
Montvale, NJ 07645

Tel: (201) 497-5993
Fax: (201) 497-5994

β -Carotene Beadlet 20% TAB-S

BC 20% TAB-S

Specification and Tests

1. Description

Red or red-brown beadlets, with white spots of food starch.

2. Identification

The retention time of principal peak conforms to which in the reference solution, as obtained in the test for Beta-carotene content.

3. Loss on drying

Max. 8.0%

Proceed according to USP<731> / Ph. Eur. 2.2.32.

4. Heavy metals (as Pb)

Max. 0.001%

Proceed according to USP <231> II

5. Arsenic (as As)

Max. 0.0003%

Proceed according to Ph. Eur.2.4.2.

6. Total carotenoid content (UV)

Min. 20.0%

1) Accurately weight approximately 70 mg of sample into a 100ml brown volumetric flask, 5 ml deionized water is added and the flask is placed into the ultrasonic bath under 60° C for 5 min. The mixture is allowed to cool down to room temperature, add 50 ml anhydrous ethanol and fill to volume with methylene chloride.

2) A volume of 10 ml of the carefully shaken fine dispersion is centrifuged for 5 minutes. Take 1ml of the clear solution and put it in a 50ml brown flask, and dilute with ethanol: cyclohexane (1:9) to the volume.

3) The absorbance is measured by spectrophotometry at the maximum wavelength of 454nm using cyclohexane as blank.

Total carotenoid content is calculated by below formula

$$\text{Total carotenoid content} = \frac{A \times 50}{W \times 2230} \times 100\%$$

Sequence No.: 0106030000024
Date: 07-01-2023
Version No.: 001

Where:

A	=	Absorbance of the measuring solution at 454 nm
W	=	Sample Weight, in g
50	=	Dilution factor
2230	=	Absorbance coefficient for beta-carotene

7. Beta-Carotene content (HPLC)

Min. 20.0%

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 to room temperature, and dilute with methanol to the 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 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, 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 into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.

Instrumental conditions

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 : Transfer an amount of Beta Carotene 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 solution: Transfer 5.0 mL of Sample stock solution into a 50-mL volumetric flask, and dilute with Diluent to volume. Pass through a membrane filter of 0.45-µm pore size.

Chromatographic system

Mode: LC

Detector: UV 448 nm

Column: 4.6-mm × 25-cm; 5-µm packing L68

Column temperature: 30°C

Flow rate: 0.6 mL/min

Injection size: 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.

Table1

Name	Relative retention time	Relative response factor
All-trans α-carotene	0.93	1.0
All-trans β-carotene	1.00	1
9-cis β-carotene	1.07	1
13-cis β-carotene	1.17	1.2
15-cis β-carotene	1.21	1.4

Suitability requirements

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 5 replicate injections, Standard solution A.

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 total Beta-carotene in the portion of Preparation taken:

$$\text{Result} = \frac{Ru \times Cs}{Rs \times Cu} \times 100$$

Ru = [(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

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

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

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

8. Particle Size

Thru #20 US	100.0%
Thru #40 US	Min. 85.0%
Thru #100 US	Max. 15.0%

9. Microbial Test

Corresponds

Proceed according to Ph. Eur.2.6.12 and Ph. Eur.2.6.13 / USP<2021> and USP<2022>.