β-CAROTENE, synthetic

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 31st JECFA (1987), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw for beta carotene, synthetic and from Blakeslea trispora, was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a(i); CI (1975) No. 40800

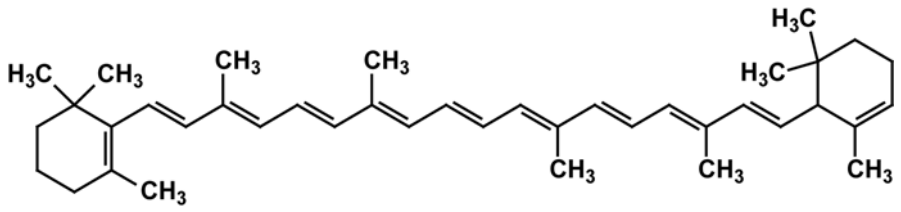
DEFINITION These specifications apply to synthetic β-carotene which consists predominantly of all-*trans*-β-carotene. Synthetic β-carotene may also contain minor amounts of *cis*-isomers and other carotenoids such as all-*trans*-retinal, β-apo-12'-carotenal, and β-apo-10'-carotenal. Commercial preparations of β-carotene intended for use in food are prepared from β-carotene meeting these specifications and are formulated as suspensions in edible oils or water-dispersible powders. These preparations may have different ratio of trans/cis isomers.

Chemical names ß-Carotene, ß,ß-carotene
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]

C.A.S. number 7235-40-7

Chemical formula $C_{40}H_{56}$

Structural formula All-*trans*-β-carotene (main compound)



Formula weight 536.88

Assay Not less than 96% total colouring matters, expressed as ß-carotene.

DESCRIPTION Red to brownish-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils.
Test for carotenoids	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.19. Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 340 nm. The ratio A_{455}/A_{340} is not lower than 15.

PURITY

Sulfated Ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than ß-carotene: Not more than 3% of total colouring matters. See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, under "General Methods, Metallic Impurities."

TESTS

PURITY TESTS

Subsidiary colouring matters

Carotenoids other than β-carotenes

Subsidiary colouring matters (carotenoids other than β-carotenes) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 453 nm
- Column: Reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room

temperature and dilute to volume with methanol. Discard after 2 days.

- Isocratic elution
- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents

- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution

Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure

Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention times for all-*trans*-β-carotene and cis-isomers are in the range of 20-25 min. The largest peak in the chromatogram corresponds to all-*trans*-β-carotene. The relative retention times of minor carotenoids and cis-isomers of β-carotene with respect to the retention time of all-*trans*-β-carotene are: all-*trans*-retinal (0.26), all-*trans*-β-apo-12'-carotenal (0.33), all-*trans*-β-apo-10'carotenal (0.34), all-*trans*-γ-carotene (0.85), all-*trans*-α-carotene (0.95), 9-cis-β-carotene (1.05), 13-cis-β-carotene (1.15) and 15-cis-β-carotene (1.18).
Integrate the areas of the peaks in the chromatogram.

Calculation

Calculate the percentage of carotenoids other than β-carotenes (%, w/w) using the following formula:

$$\text{Carotenoids other than } \beta\text{-carotenes (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\beta-\text{carotenes}}}{A_{\text{total}}} \right) \times 100$$

where

$A_{\text{total}'}$ is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and

$A_{\beta\text{-carotene}}$ is the sum of the areas of the peaks of all β-carotenes (all-*trans*-β-carotene, 9-cis-β-carotene, 13-cis-β-carotene and 15-cis-β-carotene) in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Content by Spectrophotometry, Procedure 2, using the following conditions:

Sample weight (W): 0.08 g (±0.01 g);
Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml;
Volume of the two pipets: $v_1 = v_2 = 5$ ml;
Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2500$;
Wavelength of maximum absorption: λ_{max} about 455 nm.

Calculation

Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) = = \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 455 nm; and
D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.