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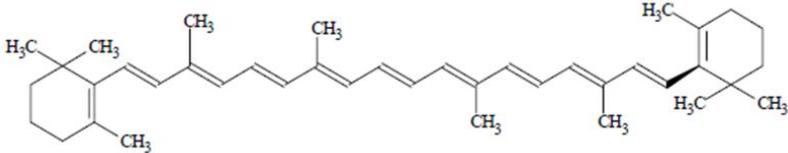
Specification Monograph prepared by the meeting of the Joint FAO/WHO  
Expert Committee on Food Additives (JECFA), 87th Meeting 2019

## **$\beta$ -CAROTENE, SYNTHETIC**

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## β-CAROTENE, SYNTHETIC

Prepared at the 87<sup>th</sup> JECFA (2019) and published in FAO Monographs 23 (2019). Superseding specifications prepared at the 74<sup>th</sup> JECFA (2011) and published in FAO Monographs 11 (2011). A group ADI of 0-5 mg/kg bw for beta carotene, synthetic and from *Blakeslea trispora*, was established at the 57<sup>th</sup> JECFA (2001).

|                    |  |
|--------------------|--|
| <b>SYNONYMS</b>    | CI Food Orange 5; INS No. 160°(i); CI (1975) No. 40800   |
| <b>DEFINITION</b>  | 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     | β-Carotene, β,β-carotene<br>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 <sub>40</sub> H <sub>56</sub>  |
| Structural formula | All-trans-β-carotene (main compound)<br>   |
| Formula weight     | 536.88   |
| Assay              | Not less than 96% total colouring matters, expressed as β-carotene.  |
| <b>DESCRIPTION</b> | 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

Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils.

Spectrophotometry  
(Vol. 4)

Determine the absorbance of the diluted sample solution used in the Method of Assay from 300 – 600nm. The spectrum shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The ratio  $A_{455}/A_{483}$  is between 1.14 and 1.18.

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 less than 15.

PURITY

Sulfated Ash (Vol. 4)

Not more than 0.1%  
Test 2 g of the sample (Method I).

Carotenoids other than  $\beta$ -carotene

Not more than 3% of total colouring matters.  
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg.

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, under "General Methods, Metallic Impurities".

**TESTS**

PURITY TESTS

Carotenoids other than  $\beta$ -carotene

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  $\mu$ m) 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-ethyl-diisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and 3 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: (4 approx.. 15°)
- Run time: 4 approx.. 35 min

#### Reagents

- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyl-diisopropyl-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*-□-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:

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

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**    **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 ( $\pm 0.01$  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:  $A_{1\text{cm}}^{1\%} = 2500$ ;

Wavelength of maximum absorption:  $\lambda_{\text{max}}$  about 455 nm.

**Calculation**

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

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

where

A is the absorbance of the twice-diluted sample solution at 455 nm; and

D is the dilution factor  $(V_2 \times V_3) / (v_1 \times v_2)$ .