β-CAROTENE from *BLAKESLEA TRISPORA*

Prepared at the 61st JECFA (2003), published in FNP 52 Add 11 (2003), superseding specifications prepared at the 57th JECFA (2001), published in FNP 52 Add 9 (2001). A group ADI with β -carotene (synthetic) of 0 - 5 mg/kg bw was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a

DEFINITION Obtained by a fermentation process using the two sexual mating types (+) and (-) of the fungus *Blakeslea trispora*. The colour is isolated from the biomass by solvent extraction and crystallised. The colouring principle consists predominantly of trans β-carotene together with variable amounts of cis isomers of β-carotene. Minor amounts of other carotenoids of which γ-carotene accounts for the major part may also be present. The only organic solvents used in the extraction and purification are ethanol, isopropanol, ethyl acetate and isobutyl acetate. The main articles of commerce are suspensions in food grade vegetable/plant oil and water dispersible powders. This is for ease of the use and to improve stability as carotenes easily oxidise.

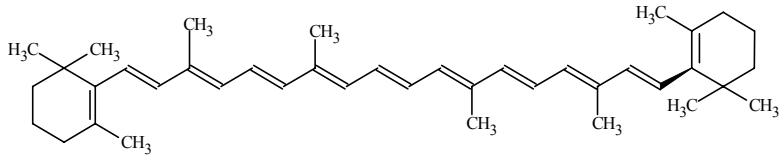
Class: Carotenoid

Chemical names: β-carotene, β,β-carotene

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

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

Structural formula



Formula weight: 536.88

Assay: Not less than 96.0% of total colouring matter (expressed as β-carotene)

DESCRIPTION Red to brownish-red crystals or crystalline powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; practically insoluble in ethanol, slightly soluble in vegetable oil.

UV/VIS absorption

Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 483 nm. The ratio 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 is not lower than 0.75.

Carotenoid

The colour of a solution of the sample in acetone disappears after successive addition of a 5% solution of sodium nitrite and 0.5 M of sulfuric acid.

PURITY

Sulfated ash (Vol. 4)

Not more than 0.2%

Carotenoids other than β-carotene

Not more than 3.0% of total colouring matters
See description under TESTS

Residual solvent (Vol. 4)

Ethanol:
Ethyl acetate: } Not more than 0.8% singly or in combination
Isopropanol: Not more than 0.1%
Isobutyl acetate: Not more than 1.0%

See description in Volume 4

Lead (Vol. 4)

Not more than 2 mg/kg
Determine using an atomic absorption 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, "Instrumental methods".

TESTS

PURITY TESTS

Carotenoids other than β-carotene

Determine by liquid chromatography (see Volume 4) using the following procedure:

Apparatus:
HPLC system equipped with
- UV/VIS detector (445 nm)
- Column heater (30°)
- Refrigerated autosampler (0-10°)
- Column: 250 mm x 4.6 mm, Vydac 218 TP54, 5 μm, or equivalent
- Solvent system: 99% methanol and 1% tetrahydrofuran containing 50 mg/l of L-ascorbic acid.
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Working conditions:
- Flow rate: 0.6 ml/min
- Injection 10 μl
- Run time: approximately 25 min

Sample preparation: Weigh 25 mg of the sample and dissolve in tetrahydrofuran. Make up to 100 ml in a volumetric flask. Dilute 1 ml of the solution to 25 ml in a volumetric flask with a ethanol:tetrahydrofuran (9:1) solution.

Results: The retention time for β-carotene (all trans isomer) is about 19 minutes corresponding to the largest peak in the chromatogram. The retention time for γ-carotene is about 20 minutes and the peak at about 22 minutes corresponds to the 13-cis isomer.
γ-carotene as a % of total β-carotene equals:

$$\frac{A_1 \times 100}{A_1 + A_2 + A_3}$$

where

A_1 is the area of the γ-carotene peak
A_2 is the area of the all-trans β-carotene peak
A_3 is the combined area of the peaks from the isomers of all-trans β-carotene

METHOD OF ASSAY Proceed as directed in Colouring matters, Total Content by Spectrophotometry (Volume 4), procedure 2 using the following conditions:

W = 0.08 g
$V_1 = V_2 = V_3 = 100$ ml
$v_1 = v_2 = 5$ ml
$A^{1\%}_{1\ cm} = 2500$
A_{max} = about 455 nm