ß-apo-8'-CAROTENAL

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw expressed as the sum of carotenoids including β-carotene, β-apo-8'-carotenal, and the methyl and ethyl esters of β-apo-8'-carotenoic acid was established at the 18th JECFA (1974).

SYNONYMS CI Food Orange 6; CI (1975) No. 40820; INS No. 160e

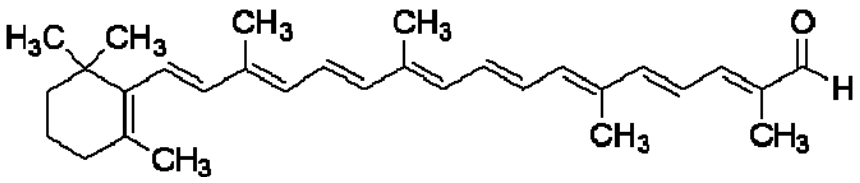
DEFINITION These specifications apply to β-apo-8'-carotenal which consists predominantly of all-*trans*- β-apo-8'-carotenal and may also contain minor quantities of other carotenoids such as all-*trans*-crocetindialdehyde, all-*trans*-β-apo-12'-carotenal and all-*trans*-β-carotene. Commercial preparations of β-apo-8'-carotenal intended for use in food are prepared from β-apo-8'-carotenal meeting these specifications and are formulated as suspensions in edible oil, emulsions and water dispersible powders. These preparations may also contain cis isomers.

Chemical names ß-Apo-8'-carotenal, 8'-apo-ß-carotene-al
2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethyl-1-cyclohexenyl)heptadeca-2,4,6,8,10,12,14,16-octaenal

C.A.S. number 1107-26-2

Chemical formula $C_{30}H_{40}O$

Structural formula *All-trans*- β-apo-8'-carotenal (main compound)



Formula weight 416.65

Assay Not less than 96% of total colouring matters

DESCRIPTION Deep violet crystals with metallic lustre 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, slightly soluble in ethanol, sparingly soluble in vegetable oils.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 461 nm and 488 nm. The ratio A_{488}/A_{461} is between 0.80 and 0.84.
Test for carotenoid	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.

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-apo-8'-carotenal: 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 ß-apo-8'-carotenal

Subsidiary colouring matters (carotenoids other than ß-apo-8'-carotenal) 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: 463 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 time for all-*trans*-ß-apo-8'-carotenal is in the range of 7-9 min and corresponds to the largest peak in the chromatogram. The relative retention times of minor carotenoids with respect to the retention time of all-*trans*-ß-apo-8'-carotenal are: all-*trans*-crocetindialdehyde (0.54); all-*trans*-β-apo-12'-carotenal (0.84); all-*trans*-β-carotene (2.55).
Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than ß-apo-8'-carotenal (%, w/w) using the following formula:

$$\text{Carotenoids other than ß - apo - 8'-carotenal (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\text{β-apo-8'-carotenal}}}{A_{\text{total}}} \right) \times 100$$

where

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

$A_{\text{ß-apo-8'-carotenal}}$ is the area of the peak of ß-apo-8'-carotenal 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 Contents 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: $A^{1\%}_{1\ cm}$ = 2640
Wavelength of maximum absorption: λ_{max} about 461nm

Calculation

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

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

where

A is the absorbance of the twice-diluted sample solution at 461 nm; and
D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.