

β -apo-8'-CAROTENOIC ACID ETHYL ESTER

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 7; CI (1975) No. 40825; INS No. 160f

DEFINITION

These specifications apply to β -apo-8'-carotenoic acid ethyl ester which consists predominantly of all-*trans*- β -apo-8'-carotenoic acid ethyl ester and may also contain minor quantities of all-*trans*- β -apo-12'-carotenal, methyl-all-*trans*- β -apo-8'-carotenoate, all-*trans*-ethyl 4'-apo- β -carotenate and all-*trans*- β -carotene. Commercial preparations of β -apo-8'-carotenoic acid ethyl ester intended for use in food are prepared from β -apo-8'-carotenoic acid ethyl ester 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

All-*trans*- β -apo-8'-carotenoic acid ethyl ester, ethyl 8'-apo- β -caroten-8'-oate, ethyl (2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohexen-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaenoate

C.A.S. number

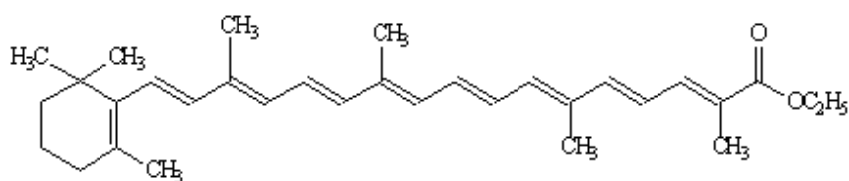
1109-11-1

Chemical formula

C₃₂H₄₄O₂

Structural formula

All-*trans*- β -Apo-8'-carotenoic acid ethyl ester (main compound)



Formula weight

460.70

Assay

Not less than 96% of total colouring matters

DESCRIPTION

Red to violet-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

<u>Solubility</u> (Vol. 4)	Insoluble in water, very slightly soluble in ethanol, slightly soluble in vegetable oils.
<u>Spectrophotometry</u> (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 449 nm and 475 nm. The ratio A_{475}/A_{449} is between 0.82 and 0.86.
<u>Test for carotenoid</u>	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

<u>Sulfated ash</u> (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
<u>Subsidiary colouring matters</u>	Not more than 3% of total colouring matters See description under TESTS
<u>Lead</u> (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

<u>Subsidiary colouring matters</u>	<p><u>Carotenoids other than β-apo-8'-carotenoic acid ethyl ester</u></p> <p>Subsidiary colouring matters (carotenoids other than β-apo-8'-carotenoic acid ethyl ester) are determined by high performance liquid chromatography (HPLC) using the following conditions:</p> <p><u>Chromatographic system</u></p> <ul style="list-style-type: none">– HPLC system equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler– Detector wavelength: 446 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-ethyl-diisopropyl-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-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 time for all-*trans*-apo-8'-carotenoic acid ethyl ester is in the range of 9-11 min and corresponds to the largest peak in the chromatogram. The relative retention times of carotenoids with respect to the retention time of all-*trans*- β -apo-8'-carotenoic acid ethyl ester are: all-*trans*- β -apo-12'-carotenal (0.73); methyl all-*trans*- β -apo-8'-carotenoate (0.97); all-*trans*-ethyl 4'-apo- β -carotenate (1.22), all-*trans*- β -carotene (2.23). Integrate the areas of the peaks in the chromatogram.

Calculation

Calculate the percentage of carotenoids other than β -apo-8'-carotenoic acid ethyl ester (% w/w) using the following formula:

$$\begin{aligned} & \text{Carotenoids other than } \beta\text{-apo-8'-carotenoic acid ethyl ester (\%, w/w)} \\ &= \left(\frac{A_{\text{total}} - A_{\beta\text{-apo ester}}}{A_{\text{total}}} \right) \times 100 \end{aligned}$$

where

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

$A_{\beta\text{-apo-ester}}$ is the area of the peak of β -apo-8'-carotenoic acid ethyl ester 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: $A_{1\%}^{1\text{cm}} = 2550$

Wavelength of maximum absorption: λ_{max} about 449 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 449 nm; and
D is the dilution factor $(V_2 \times V_3) / (v_1 \times v_2)$.