

β-apo-8'-CAROTENOIC ACID ETHYL ESTER

Prepared at the 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). A group ADI of 0-5 mg/kg bw expressed as the sum of the carotenoids:beta-carotene, beta-apo-8'-carotenal was established at the 18th JECFA (1974)

SYNOMYS

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

DEFINITION

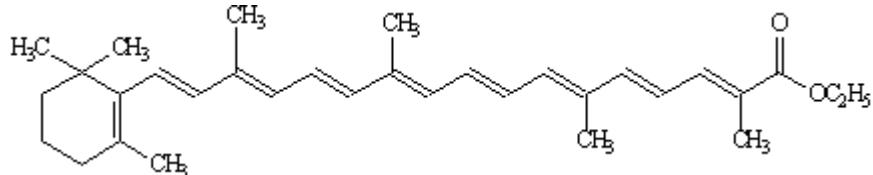
Specifications apply to predominantly all trans (Z) isomer of β-apo-8'-carotenoic acid ethyl ester together with minor amounts of other carotenoids; diluted and stabilized forms are prepared from β-apo-8'-carotenoic acid ethyl ester meeting these specifications and include solutions or suspensions of β-apo-8'-carotenoic acid ethyl ester in edible fats or oils, emulsions and water dispersible powders; these preparations may have different cis/trans isomer ratios. The analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms (appropriate methods should be available from the manufacturer).

Chemical names β-Apo-8'-carotenoic acid ethyl ester, ethyl 8'-apo-β-caroten-8'-oate.

C.A.S. number 1109-11-1

Chemical formula C₃₂H₄₄O₂

Structural formula



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

Solubility (Vol. 4) Insoluble in water, very slightly soluble in ethanol, slightly soluble in vegetable oils, soluble in chloroform

Spectrophotometry Determine the absorbance of the sample solution (See Method of Assay) at 449 nm and 475 nm. The ratio A₄₇₅/A₄₄₉ is between 0.82 and 0.86.

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 1 N sulfuric acid.

Carr-Price reaction

A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS.

PURITY

Sulfated ash (Vol. 4)

Not more than 0.1%

Test 2 g of the sample (Method I)

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."

Subsidiary colouring matter

Not more than 3% of total colouring matters

See description under TESTS

TESTS

PURITY TESTS

Subsidiary colouring matter

Carotenoids other than β -apo-8'-carotenoic acid ethyl ester: Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 μ l of this solution as a streak 2 cm from the bottom of a TLC-plate (Silicagel 0.25 mm).

Pretreat the thin-layer plate by soaking in a tank with 3% KOH in methanol so that it is completely wetted. Then dry the plate for 5 min in the air and activate for 1 h at 110° in an oven. Let cool over CaCl_2 and keep in a desiccator over CaCl_2 .

Immediately after applying the carotenoid solution to the plate, develop the chromatogram with n-hexane/chloroform/ethylacetate (70+20+10) in a saturated chamber suitably protected from light, until the solvent front has moved 10 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silicagel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (solution 1). Separately remove the silica gel of the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (solution 2). Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (solution 3).

Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum in chloroform at about 455 nm, using chloroform as a blank.

Calculation

Carotenoids other than β -apo-8'-carotenoic acid ethyl ester (%) =

$$\frac{A_2 \times 10}{A_3}$$

where

A₂ = absorbance of Solution 2

A₃ = absorbance of Solution 3

METHOD OF ASSAY

Weigh accurately about 80 mg of the sample and proceed as directed under *Total Content by Spectrophotometry* in Volume 4

Absorbitivity (a) = 2550

Approximate wavelength of maximum absorption = 449 nm.