ZEAXANTHIN (SYNTHETIC)

Prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004) and in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0 – 2 mg/kg bw for lutein and zeaxanthin (synthetic) was established at the 63rd JECFA (2004).

SYNONYMS INS No. 161h(i)

DEFINITION The synthetic all-trans isomer of zeaxanthin is produced by the Wittig condensation from synthetic intermediates commonly used in the production of other carotenoids used in foods.

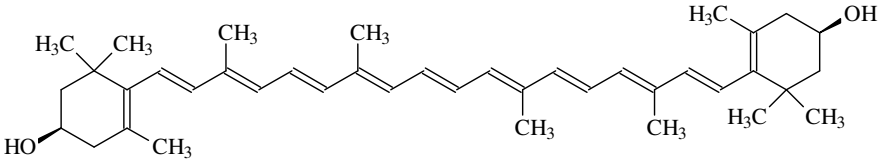
Chemical Names (all-E)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene-3-ol]

3R,3'R-β, β -Carotene-3,3'-diol

CAS. number 144-68-3

Chemical formula $C_{40}H_{56}O_2$

Structural formula



Formula weight 568.9

Assay Not less than 96%

DESCRIPTION Orange-red crystalline powder, with little or no odour

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Sparingly soluble in chloroform, practically insoluble in water and ethanol

Test for carotenoid The colour of the solution of the sample in acetone disappears after successive additions of a 5 % solution of sodium nitrite and 1N sulfuric acid

Spectrophotometry (Vol. 4)	An ethanol solution of the sample shows maximum absorption between 450 and 454 nm
PURITY	
Loss on drying (Vol. 4)	Not more than 0.2 % (80° under reduced pressure for 18 h in the presence of P_2O_5)
cis-Zeaxanthins	Not more than 2.0 % See description under METHOD OF ASSAY
12'-Apo-zeaxanthinal, diatoxanthin, parasilo-xanthin	Not more than 1.1 % combined See description under METHOD OF ASSAY
Triphenyl phosphine oxide (TPPO) (Vol. 4)	Not more than 0.01%
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 methods described in Volume 4.

METHOD OF ASSAY

The HPLC method of assay is designed to determine *trans*-zeaxanthin, the *cis*-isomers of zeaxanthins and zeaxanthin-related impurities: 12'-apo-zeaxanthinal, parasiloxanthin, and diatoxanthin. (NOTE: All solvents should be HPLC grade.)

Standards

Trans-zeaxanthin, 12´-apo-zeaxanthinal, and diatoxanthin. (All *trans*-zeaxanthin, 12´-apo-zeaxanthinal, and diatoxanthin available from DSM Nutritional Products, Kaiseraugst, Switzerland. All-*trans*-zeaxanthin is also available from Fluka, Buchs, Switzerland).

Standard solutions:

Solution 1: Accurately weigh 34 to 36 mg of 12'-apo-zeaxanthinal and transfer to a 100-ml volumetric flask. Add tetrahydrofuran to dissolve the substance and bring to volume.

Solution 2: Accurately weigh 34 to 36 mg of diatoxanthin and transfer to a 100-ml volumetric flask. Add tetrahydrofuran to dissolve the substance and bring to volume.

Working standard: Accurately weigh 69.0 to 71.0 mg of *trans*-zeaxanthin and transfer to a 100-ml volumetric flask. Add 50 ml of tetrahydrofuran, 1 ml of standard solution 1, and 1 ml of standard solution 2. Bring to volume with tetrahydrofuran.

Sample solution:
Accurately weigh 69.0 to 71.0 mg of sample and dissolve in 100 ml of tetrahydrofuran.

Mobile phase:
In a 2000-ml volumetric flask containing a small quantity of hexane, add 400 ml of ethyl acetate, 20 ml of 2-methoxyethanol, and 2.0 ml of *N*-ethyldiisopropylamine. Bring to volume with hexane.

Chromatography apparatus and conditions:

Column:	Stainless steel; 250 x 4 mm
Column temperature:	25°
Stationary phase:	Spherisorb Si, 3 µm or similar
Flow:	Flow 1.0 ml/min
Detector:	VIS 450 nm
Injection:	2.0 µl
Run time:	35 min

Procedure:
Inject a 2.0 µl aliquot of the Working standard and measure the area of the peaks for *trans*-zeaxanthin, 12´-apo-zeaxanthinal, and diatoxanthin. Inject a 2.0 µl aliquot of the sample solution and measure the areas of the peaks for *trans*-zeaxanthin, *cis*-isomers of zeaxanthins, 12´-apo-zeaxanthinal, parasiloxanthin, and diatoxanthin. Typical retention times and relative retention times are shown in the table below.

Substance	Relative retention time*	Approx. absolute retention time [min]
trans-zeaxanthin	1.00	17.7
cis isomers of zeaxanthin	1.38 – 1.46	24.4 – 25.8
12´-apo-zeaxanthinal	0.46	8.2
parasiloxanthin	0.96	17.0
diatoxanthin	1.16	20.5

* in relation to trans-zeaxanthin

Calculation:
Calculate the percentage content of *trans*-zeaxanthin in the sample using the equation below:

$$(\%) = \frac{A_{(s)} \cdot W_{(R)} \cdot P_{(R)} \cdot 100}{A_{(R)} \cdot W_{(s)}}$$

Where:

- $A_{(S)}$ is the peak area of substance to be determined in the sample solution
- $W_{(R)}$ is the weight (mg) of substance in the Working standard
- $P_{(R)}$ is the purity of substance (0.98 if purity is 98%) in the Working standard
- $A_{(R)}$ is the peak area of substance in the Working standard
- $W_{(S)}$ is the weight (mg) of the sample in the sample solution

To calculate the percentage of the contents of 12´-apo-zeaxanthinal, and diatoxanthin use the equation above and the corresponding weights and purity for each substance.

Cis-isomers of zeaxanthin and parasiloxanthin are not included in the standard solutions. However, their absorptivities at the wavelength employed in the method are the same as the absorptivity for *trans*-zeaxanthin. Their percentage contents can therefore be calculated using the above formula considering their weights to be the same as that of *trans*-zeaxanthin and their purities ($P_{(R)}$) to be equal to one (100% purity = 1).