ZEAXANTHIN (SYNTHETIC)

New specifications prepared at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). A group ADI of 0 – 2 mg/kg bw for lutein and synthetic zeaxanthin was established at the 63rd JECFA (2004).

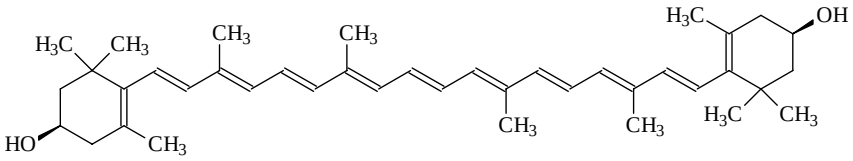
DEFINITION These specifications apply to synthetic all-trans isomer of zeaxanthin that is produced by the Wittig reaction from the raw materials that are commonly used in the production of other carotenoids with application in foods. Minor quantities of cis-zeaxanthins and byproducts 12'-apo-zeaxanthinal, parasiloxanthin diatoxanthin and triphenyl phosphine oxide may be present in the final product.

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

C.A.S. number 144-68-3

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

Structural formula



Formula weight 568.89

Assay Not less than 96.0% and not more than 101.0%

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

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

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

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 at 450 to 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 1.0 %
See description under METHOD OF ASSAY

Triphenyl phosphine oxide (TPPO)

Not more than 100 mg/kg
See description under TESTS

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 methods described in Volume 4, "Instrumental Methods".

TESTS

PURITY TESTS

Triphenyl phosphine oxide (TPPO)

Determine by HPLC using the following conditions:

Calibration solution:
10 mg/l TPPO (99 %) in tetrahydrofuran.
Test solution:
Dissolve 950 to 1000 mg of sample in 100 ml of tetrahydrofuran.
Mobile phase:
Isopropanol : hexane (1 : 20.2)
Apparatus:
Column: Stainless steel; 150 x 4.6 mm
Stationary phase: Supelcosil LC-Si, 5 µm or similar
Pump: Flow 1.5 ml/min; pressure 35 to 40 bar
Detector: UV 210 nm
Injection: 50 µl

Results:
Run time: 10 min
The retention time for TPPO is approximately 8.1 min.

Calculation:

$$\% \text{ TPPO} = \frac{A_s \times W_c \times 0.99 \times 100 \times 100}{A_c \times W_s \times 1000}$$

Where
A_s = peak area of the sample solution
A_c = peak area of the calibration solution
W_c = weight of TPPO (99 %) in mg
W_s = weight of the sample in mg

METHOD OF ASSAY

The HPLC method of assay is designed to determine *trans*-zeaxanthin, *cis*-zeaxanthins and zeaxanthin related impurities: 12'-apo-zeaxanthinal, parasiloxanthin and diatoxanthin.

Calibration solutions:
Solution 1: Accurately weigh 34 to 36 mg of 12'-apo-zeaxanthinal and dissolve in 100 ml of tetrahydrofuran.
Solution 2: Accurately weigh 34 to 36 mg of diatoxanthin and dissolve in 100 ml of tetrahydrofuran.
Solution 3: Accurately weigh 69.0 to 71.0 mg of zeaxanthin. Add 50 ml of tetrahydrofuran, 1 ml of calibration solution 1, and 1 ml of calibration solution 2. Bring to volume (100 ml) with 48 ml tetrahydrofuran.

Test 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.

Apparatus:

Column:	Stainless steel; 250 x 4 mm
Column temperature:	25°
Stationary phase:	Spherisorb Si, 3 µm or similar
Pump:	Flow 1.0 ml/min; pressure 85 bar
Detector:	VIS 450 nm
Injection:	2.0 µl
Run time:	35 min

Results:
The retention times for *trans*-zeaxanthin and *cis*-zeaxanthins are approximately 17.7 and 24.4 to 25.8 min, respectively.
The retention times for the by-products 12'-apo-zeaxanthinal, parasiloxanthin, and diatoxanthin are approximately 8.2, 17.0, and 20.5 min, respectively.
For *trans*-zeaxanthin, *cis*-zeaxanthins, and parasiloxanthin perform external standardization with the zeaxanthin response factor.
For 12'-apo-zeaxanthinal perform external standardization with the 12'-apo-zeaxanthinal response factor.
For diaxanthin perform external standardization with the diaxanthin response factor.

Substance	*Response Factor*	*Relative Response factor related to trans-Zeaxanthin at 450 nm*
trans-Zeaxanthin	$4.671e^{-10}$	1
12'-Apo-zeaxanthinal	$1.134e^{-9}$	2.428
Diatoxanthin	$5.588e^{-10}$	1.962
Parasiloxanthin	$4.671e^{-10}$	1
cis-Zeaxanthins	$4.671e^{-10}$	1

Calculation:

$$\% \text{ substance to be determined} = \frac{A_s \times RFi \times 100}{W_s}$$

Where:

As = peak area of the substance to be determined in the test solution
RFi = response factor of the substance to be determined in the test solution
Ws = weight of the sample in mg

with: $$RFi = \frac{W_r \times C_r}{A_r \times 100}$$

Where:

Wr = weight of the reference substance from the calibration in mg
Cr = content of the reference substance in percent
Ar = peak area of the reference substance from the calibration