LYCOPENE (SYNTHETIC)

New specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). A group ADI "not specified" for lycopene from all sources was established at the 71st JECFA (2009).

SYNONYMS INS 160d(i)

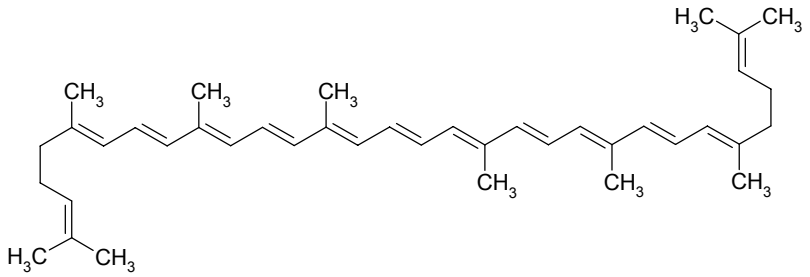
DEFINITION Synthetic lycopene is produced by the Wittig condensation of synthetic intermediates commonly used in the production of other carotenoids used in food. Synthetic lycopene consists predominantly of all-*trans*-lycopene together with 5-*cis*-lycopene and minor quantities of other isomers. Commercial lycopene preparations intended for use in food are formulated as suspensions in edible oils or water-dispersible powders and are stabilised with antioxidants.

Chemical names
Ψ,*Ψ*-carotene
all-*trans*-lycopene
(all-E)-lycopene
(all-E)-2,6,10,14,19,23,27,31-octamethyl-2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene

CAS number 502-65-8

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

Structural formula



Formula weight 536.9

Assay Not less than 96% total lycopenes; not less than 70% all-*trans*-lycopene

DESCRIPTION Red crystalline powder

FUNCTIONAL USES Colour, nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, freely soluble in chloroform

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

Solution in chloroform	A 1% solution is clear and has intensive red-orange colour
Spectrophotometry (Vol. 4)	A solution in hexane shows an absorption maximum at approximately 470 nm

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (40°, 4 h at 10 mm Hg)
Lead (Vol. 4)	Not more than 1 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 principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").
Apo-12'-lycopenal	Not more than 0.15% See description under TESTS
Triphenyl phosphine oxide (TPPO) (Vol. 4)	Not more than 0.01%

TESTS

PURITY TESTS

Apo-12'-lycopenal

Determine by HPLC using the following conditions:

Reagents (Note: all solvents should be HPLC-grade)
Hexane
Triethylamine (TEA)
Tetrahydrofuran (THF)
Toluene stabilised with BHT (0.5 g BHT in 1000 ml toluene)
Apo-12'-lycopenal (also known as lycopene C_{25}-aldehyde) standard (available from DSM Nutritional Products)

Apparatus
HPLC system with a suitable pump, injector, and integrator
Column: Stainless steel (200x4.0 mm)
Stationary phase: Nucleosil Si 100 3 µm (Macherey-Nagel or equivalent)
Detector: UV/VIS or VIS

HPLC conditions
Flow: 2.0 ml/min
Injection volume: 5.0 µl
Pressure: approx. 135 bar
Detection: 435 nm
Mobile phase: A – hexane
B – Hexane:TEA (99.9:0.1) (v/v)
C – Hexane:THF (80:20) (v/v)

Gradient

Time, min	A%	B%	C%
0	80	20	0
16	60	20	20
22	40	20	40
24.5	80	20	0

Run time
Approximately 25 min.

Standard solution
Accurately weigh between 14.5 and 15.5 mg of the *apo*-12'-lycopenal standard into a 50-ml volumetric flask. Dissolve in toluene stabilised with BHT and make up to volume. Transfer 2 ml of the solution into 100-ml volumetric flask and add toluene stabilised with BHT to volume.

Sample solution
Accurately weigh between 29.0 and 31.0 mg of the sample into a 10-ml volumetric flask and dissolve and dilute to volume with toluene stabilised with BHT. Put the solution in an ultrasonic bath for 10 min.

Results
The retention time of a*po*-12'-lycopenal is approximately 14 min. The relative retention time of *apo*-12'-lycopenal with respect to all-*trans*-lycopene is 1.6.

Calculation

$$Apo\text{-}12'\text{-lycopenal}\,(\%) = \frac{A_S \times W_{St} \times 10}{A_{St} \times W_S \times 2500} \times 100$$

where
A_S is the peak area of the sample;
A_{St} is the peak area of the standard;
W_{St} is the weight of the standard (mg);
W_S is the weight of the sample (mg);
10 is the volume of the volumetric flask in which the sample was dissolved (ml); and
2500 is the volume of the volumetric flask in which the standard was dissolved (50 ml) multiplied by dilution (50).

METHOD OF ASSAY Determine total lycopenes and all-*trans*-lycopene by HPLC using the following conditions:

Reagents (Note: all solvents should be HPLC-grade)
Hexane
Tetrahydrofuran stabilised with 0.025% BHT
N-Ethyl-diisopropylamine
Lycopene standard (purity 95% or higher; available from CaroteNature GmbH)

Apparatus
Spectrophotometer with a 1-cm cuvette
HPLC system with a suitable pump, injector, thermostated column compartment, and integrator
Column: Two serially-connected two stainless steel columns (250x4.0 mm)
Stationary phase: Nucleosil 300-5, 5 µm (Macherey-Nagel or equivalent)
Detector: UV/VIS or VIS

HPLC conditions

Flow rate: 0.8 ml/min
Injection volume: 20µl
Pressure: approx. 80 bar
Column temperature: 20°
Detection: 470 nm
Mobile phase: 0.15% solution of N-ethyl-diisopropylamine in hexane (v/v)
Run time: 30 min

HPLC standard solution
Accurately weigh between 5.5 and 6.5 mg of the lycopene standard into a 100-ml volumetric flask. Dissolve in 5 ml of tetrahydrofuran stabilised with BHT and make up to volume with hexane. This is a standard solution for the HPLC assay.

Spectrophotometric standard solution
Transfer 5.0 ml of the HPLC standard solution into a 100-ml volumetric flask and make up to volume with hexane. This is a standard solution for the spectrophotometric determination of lycopene in the lycopene standard.

Sample solution
Accurately weigh between 4.5 and 5.5 mg of the sample into a 100-ml volumetric flask. Dissolve in 5 ml of tetrahydrofuran stabilised with BHT and make up to volume with hexane.

Spectrophotometric determination of lycopene
Measure the absorbance of the spectrophotometric standard solution in a 1-cm cuvette at the wavelength of maximum absorption (approximately 470 nm). Use hexane as the blank.

Calculation

$$C_{St}\,(\text{mg/l}) = \frac{A \times 10000}{3450}$$

where
C_{St} is the lycopene concentration in the spectrophotometric standard solution (mg/l);
A is absorbance at the wavelength of maximum absorption;
3450 is the specific absorbance $A_{1cm}^{1\%}$ of all-*trans*-lycopene in hexane; and
10000 is the scaling factor.

HPLC analysis
Repeatedly inject 20 µl of the HPLC standard solution. Record the total peak area of all detected lycopene isomers (exclude the solvent peak). Calculate the mean peak area from repeated injections and calculate the lycopene response factor (RF) according to the formula:

$$RF = \frac{A_{St}}{C_{St} \times 20}$$

where
RF is the response factor of lycopene (AU x l/mg);
A_{St} is the mean peak area of all lycopene peaks (AU);
C_{St} is the concentration of lycopene in the spectrophotometric standard solution (mg/l); and
20 is the dilution factor used in the preparation of the spectrophotometric standard solution from the HPLC standard solution.

Inject the sample solution and record the peak areas of lycopene isomers.

Results
Retention times

Lycopene isomer	Relative retention time*	Absolute retention time (approx.)
13-*cis*-lycopene	0.6	14 min
9-*cis*-lycopene	0.8	19 min
All-*trans*-lycopene	1.0	22 min
5-*cis*-lycopene	1.1	24 min

* relative to all-*trans*-lycopene

Calculations

Calculate the content of total lycopenes according to the formula:

$$\text{Total lycopenes}\,(\%) = \frac{(A_{trans} + A_{5cis} + A_{9cis} + A_{13\,cis} + A_{xcis}) \times 0.1}{RF \times W_S} \times 100$$

Where:
A_{trans} is the peak area of all-*trans*-lycopene (AU);
A_{5cis}, A_{9cis}, and A_{13cis} are the peak areas of 5*cis*-, 9*cis*-, and 13*cis*-lycopene (AU);
A_{xcis} is the peak area of other *cis* isomers, if detected (AU);
0.1 is the volume of the flask in which the sample was dissolved (l);
RF is the response factor of lycopene (AU x l/mg); and
W_S is the weight of the sample (mg).

Calculate the content of all-*trans*-lycopene as follows:

$$\text{All - }trans\text{ - lycopene}\,(\%) = \frac{A_{trans} \times 0.1}{RF \times W_S} \times 100$$