LYCOPENE (SYNTHETIC)

New specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). A group ADI of 0-0.5 mg/kg bw for synthetic lycopene and lycopene from Blakeslea trispora was established at the 67th JECFA (2006).

SYNONYMS INS 160d(i)

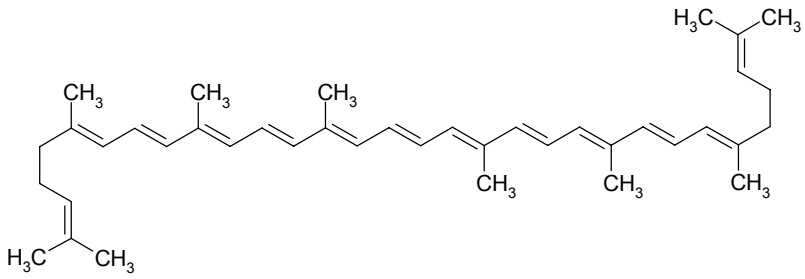
DEFINITION Synthetic lycopene is produced by the Wittig condensation of synthetic intermediates commonly used in the production of other carotenoids used in food. Synthetic lycopene consists predominantly of all-*trans*-lycopene together with 5-*cis*-lycopene and minor quantities of other isomers. Commercial 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 the principles of the methods described in Volume 4.
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)

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:

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

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 $A^{1\%}_{1cm}$ of all-*trans*-lycopene in hexane

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)

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)

W_s is the weight of the sample (mg)

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

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