

## LYCOPENE FROM *BLAKESLEA TRISPORA*

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

### SYNONYMS

INS 160d(iii)

### DEFINITION

Lycopene from *Blakeslea trispora* is extracted from the fungal biomass and purified by crystallization and filtration. It consists predominantly of all-*trans*-lycopene. It also contains minor quantities of other carotenoids. Isopropanol and isobutyl acetate are the only solvents used in the manufacture. Commercial lycopene preparations intended for use in food are formulated either as suspensions in edible oils or as water-dispersible powders and are stabilised with antioxidants.

### Chemical names

$\Psi, \Psi$ -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

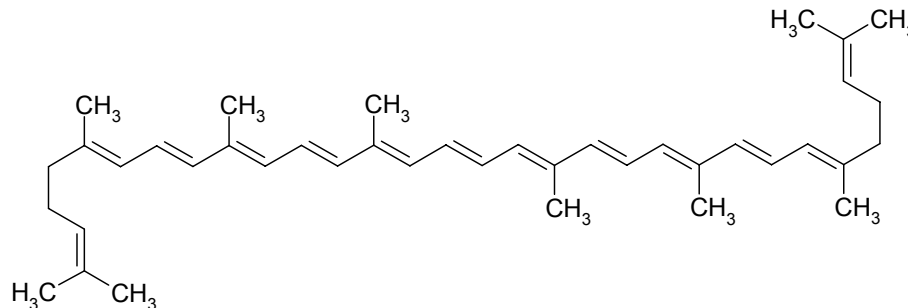
### CAS number

502-65-8

### Chemical formula

C<sub>40</sub>H<sub>56</sub>

### Structural formula



### Formula weight

536.9

### Assay

Not less than 95% total lycopenes; not less than 90% all-*trans*-lycopene

### DESCRIPTION

Red crystalline powder

### FUNCTIONAL USES

Colour

### 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 nitrite 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

- Other carotenoids Not more than 5%  
See description under METHOD OF ASSAY
- Loss on drying (Vol. 4) Not more than 0.5% (40°, 4 h at 20 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").
- Residual solvents (Vol. 4) Isopropanol: Not more than 0.1%  
Isobutyl acetate: Not more than 1.0%

#### METHOD OF ASSAY

The HPLC method of assay is suitable for determination of total lycopenes (*all-trans*-lycopene and *cis*-lycopene isomers), *all-trans*-lycopene, and other carotenoids. (Note: the predominant *cis* isomer detected in lycopene from *B. trispora* is 13-*cis*-lycopene.)

##### Reagents (Note: all solvents should be HPLC-grade)

Acetonitrile  
Methanol  
Acetone  
Hexane  
Methylene chloride  
Lycopene standard (purity 95% or higher; available from Vitatene S.A.)

##### Apparatus

VIS or UV/VIS spectrophotometer with a 1-cm light path optical cell  
HPLC system with either a VIS or UV/VIS detector or a suitable diode array detector, injector, column oven, and integrator  
Column: Vydac 218 TP54 5 m (4.6x250 mm) or equivalent

##### HPLC conditions

Mobile phase: acetonitrile/methanol (40:60)  
Flow rate: 1 ml/min  
Detection: 470 nm  
Injection volume: 10 µl  
Column temperature: 30°  
Injector temperature: 10°  
Run time: 15 min

##### Standard solution

Weigh accurately about 25 mg lycopene standard into a 100-ml volumetric flask. Dissolve in 10 ml of methylene chloride and add hexane to volume. Pipet 1 ml of the above solution into a 50-ml volumetric flask and add acetone to volume.

### Sample solution

Prepare as the standard solution.

### HPLC analysis

Chromatograph the standard solution. The retention time of all-*trans*-lycopene is approximately 11.5 to 12.5 min. The relative retention time of 13-*cis*-lycopene with respect to all-*trans*-lycopene is 1.25. The relative retention times for other carotenoids with respect to all-*trans*-lycopene are 1.2 for  $\beta$ -carotene and 1.1 for  $\gamma$ -carotene.

Record the total peak area of all-*trans*-lycopene and *cis*-lycopene isomers and calculate the response factor (RF) for lycopene as follows:

$$RF = \frac{A_{st} \times 5000}{W_{st} \times P_{st}}$$

where

RF is the response factor for lycopene (AU ml/mg);

$A_{st}$  is the total lycopene (all-*trans*-lycopene + *cis*-lycopene isomers) peak area;

5000 is the volume of the volumetric flask in which the standard was dissolved (100 ml) multiplied by dilution (50);

$W_{st}$  is the weight of the standard (mg); and

$P_{st}$  is the purity of the standard expressed as a proportion of lycopene in the lycopene standard (determined as described under Standard purity determination).

Chromatograph the sample solution and record the following peak areas:

A1 – all-*trans* lycopene

A2 – total lycopene (all-*trans*-lycopene + *cis*-lycopene isomers)

A3 – other carotenoids

A4 – all carotenoids (all-*trans*-lycopene + *cis*-lycopene isomers + other carotenoids)

### Results

Calculate the % of total lycopenes, all-*trans*-lycopene, and other carotenoids as follows:

$$\text{Total lycopenes (\%)} = \frac{A_2 \times 5000}{W \times RF} \times 100$$

$$\text{All-}i\text{trans}\text{-lycopene (\%)} = \frac{A_1}{A_2} \times 100$$

$$\text{Other carotenoids (\%)} = \frac{A_3}{A_4} \times 100$$

where

W is the sample weight (mg);

RF is the response factor (AU ml/mg); and

5000 is the volume of the volumetric flask in which the standard was dissolved (100 ml) multiplied by dilution (50).

### Standard purity determination

Accurately weigh about 20 mg of the lycopene standard into a 100-ml volumetric flask. Dissolve in 10 ml of methylene chloride and add hexane to volume. Pipet 1 ml of the solution into a 100-ml volumetric flask and add hexane to volume. Measure the absorbance in a 1-cm optical cell at the wavelength of maximum absorption (approximately 470 nm). Use hexane as the blank.

### Calculation

$$P_{st} = \frac{A_{max} \times 10000}{345 \times W_{st}}$$

where

$P_{st}$  is the purity of the lycopene standard calculated as a proportion of lycopene in the lycopene standard (NOTE:  $P_{st}$  equals 1 for a 100% pure standard and is less than 1 for a standard with purity below 100%);

$A_{max}$  is the absorbance at the wavelength of maximum absorption;

$W_{st}$  is the weight of the standard (mg);

10000 is the volume of the volumetric flask in which lycopene was dissolved (100 ml) multiplied by dilution (100); and

345 is the absorptivity of lycopene in hexane.