LYCOPENE EXTRACT FROM TOMATO

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI "not specified" for lycopene from all sources was established at the 71st JECFA (2009).

SYNONYMS Lycopene (tomato); INS 160d(ii)

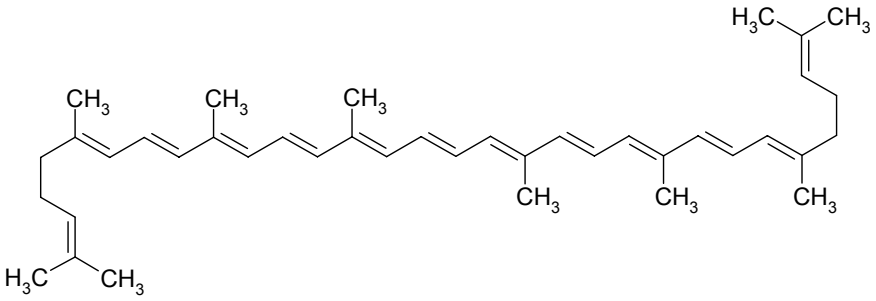
DEFINITION Lycopene extract from tomatoes is obtained by ethyl acetate extraction of the pulp of ripe red tomatoes (*Lycopersicon esculentum* L.) with subsequent removal of the solvent. The major colouring principle in tomato extract is lycopene; however, minor amounts of other carotenoid pigments may also be present. The product also contains oils, fats, waxes, and flavour components naturally occurring in tomatoes.

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

C.A.S. number 502-65-8 (lycopene)

Chemical formula $C_{40}H_{56}$ (lycopene)

Structural formula All-*trans*-lycopene, the major colouring principle



Formula weight 536.85 (lycopene)

Assay Not less than 5% and not more than 15% total lycopenes.
Not less than 6.5% and not more than16.5% total carotenoids (calculated as lycopene)

DESCRIPTION Dark-red viscous liquid

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in ethyl acetate and n-hexane; partially soluble in ethanol and acetone; and insoluble in water.
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 1 M sulfuric acid.
Spectrophotometry (Vol. 4)	A solution in n-hexane shows an absorption maximum at approximately 472 nm.

PURITY

Sulfated Ash (Vol. 4)	Not more than 1.0%, using a sample of 1-2 g
Residual Solvents	Ethyl acetate: Not more than 50 mg/kg See description under TESTS
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 method described in Volume 4 (under "General methods, Metallic Impurities").
Arsenic (Vol. 4)	Not more than 3 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 method described in Volume 4 (under "General methods, Metallic Impurities").

TESTS

PURITY TESTS

Residual solvents

Ethyl acetate is determined by headspace gas chromatography.

Chromatographic system

- Detector: flame ionization
- Column*:* Megabore fused silica (30 m x 0.53 mm I.D), coated with a 3 µm-film of 5% diphenyl-95% dimethyl polysiloxane
- Carrier gas: nitrogen
- Flow rate: 4 ml/min
- Injector temperature: 180°
- Detector temperature: 230°
- Oven temperature: 5 min at 73°; to 160° at 25°/min; then 1 min at 160°
- Injection mode: splitless 1:6
- Run time: 9.5 min

Ethyl acetate stock solutions

- *Solution A* (10,000 mg/kg): Accurately weigh 500 mg of ethyl acetate to a flask, and bring accurately to 50.00 g with diethylphthalate (use an ultrasonic bath to dissolve).The solution is stable at least for two months at room temperature.

- *Solution B* (100 mg/kg): Accurately weigh 500 mg of Solution A to a flask, and bring accurately to 50.00 g with diethylphthalate (use an ultrasonic bath to dissolve). The solution is stable at least for two months at room temperature.

Ethyl acetate standard solutions

- *Solution C (5 mg/kg):* Accurately weigh 500 mg of Solution B into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution D (10 mg/kg):* Accurately weigh 1000 mg of Solution B, into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution E (17.5 mg/kg):* Accurately weigh 1750 mg of Solution B into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution F (25 mg/kg):* Accurately weigh 2500 mg of Solution B into a 20 mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

NOTE: The vials are pre-weighted.

Sample solution

Select a representative sample of 30 g sample from the lot. The sampling should be done after heating the sample lot to 40-50° and extensive mechanical stirring. Warm the sample to 50° in a water bath, mix well with a glass rod or a spatula and weigh accurately 5000 mg of the sample into a 20-mm headspace vial. Bring the weight of the sample accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial. Mix well using a magnetic stirrer.

Procedure

Place the four standard solutions (C, D, E and F) and the sample solution in a thermostatic water bath (70°) for exactly 2 h, stirring each one for 1 min every 30 min. Inject 1000 µl of each standard solution into the head-space gas chromatograph-FID system. Record the peak area and calculate the mean ratio of the standard concentration to peak area based on concentrations and peak areas of standard solutions C, D, E, and F. Inject 1000 µl of each sample solution, record the peak area and calculate the concentration of the ethyl acetate (mg/kg), using the equation:

$$\text{Ethyl acetate (mg/kg)} = A_S \times \left(\frac{C_{ST}}{A_{ST}} \right) \times \frac{W_{tw}}{W_s}$$

where

A_s is the measured peak area of the sample solution;
(C_{ST}/A_{ST}) is the mean ratio of the standard concentration to peak area based on concentrations and peak areas of standard solutions C, D, E, and F (mg/kg);
W_{tw} is the total weight of the sample solution (g); and
W_s is the sample weight (g).

METHOD OF ASSAY Total lycopenes are determined by HPLC. Total carotenoids are determined spectrophotometrically.

Total lycopene and Total carotenoids

TOTAL LYCOPENES

Reagents

- Dichloromethane (HPLC-grade)
- Acetonitrile (HPLC-grade)
- Methanol (HPLC-grade)
- n-Hexane (HPLC-grade)
- BHT (2,6 di-tert-butyl -4-methylphenol) (A.R.)
- Petroleum ether (spirit) b.p. 60-80°(A.R.)
- Ethanol (A.R.)
- N-ethyl-diisopropylamine
- All-*trans*-lycopene standard (purity 96% or higher, available from Lycored, P.O.B. 320, Industrial Zone, Beer-Sheva, 84102, Israel)

Chromatographic system

- HPLC system with a UV/VIS detector or a diode array detector, auto sampler or injector
- Detector: 472 nm
- Column: Select B (RP-C8) (250 x 4.6 mm, 5 µm) Merck no. 50984 or equivalent
- Mobile phase: acetonitrile:methanol:dichloromethane:n-hexane:N-ethyl-diisopropylamine 850:100:25:25:0.5 (v/v/v/v/v). Mix well and sonicate for 3-4 min in an ultrasonic bath
- Flow rate: 0.7 ml/min
- Injection volume: 10 µl
- Run time: 12 min

Diluent solution

Transfer 0.5 g BHT, 600 ml acetonitrile, 100 ml methanol, 150 ml dichloromethane and 150 ml n-hexane into a 1000-ml bottle. Mix well and sonicate for 3-4 min in an ultrasonic bath.

Lycopene standard stock solution (500 mg/l)

Weigh accurately (to ± 0.1 mg) about 50 mg all-*trans*-lycopene standard into a 100-ml volumetric flask and add 100 mg of α-tocopherol and 100 mg of BHT. Add toluene to volume and sonicate 1-2 min, mix well. Dispense to 8-ml amber vials. The solution is stable for six months when stored at -18°.

Lycopene standard solutions

Take one vial of the Lycopene standard stock solution and warm to 50° in a water bath for several minutes, shaking the solution occasionally to ensure that the lycopene particles are completely dissolved. Transfer 3 ml of this solution to a 25-ml amber volumetric flask and add the Diluent solution to volume and mix (Solution A). Take another vial of the Lycopene standard stock solution and treat as above. Transfer 4 ml of this solution to a second 25-ml amber flask and add the Diluent solution to volume and mix (Solution B). Solutions A and B are stable for at least 3 weeks if held at -18°. Prior to each use, determine spectrophotometrically the lycopene concentration in each solution (See Standardization of the Lycopene standard solutions).

BHT solution (5000 mg/l)

Weigh 2.5 g BHT into a 500-ml storage bottle and add 500 ml dichloromethane. Keep the solution protected from light. This solution is stable for 3 months.

Sample solutions

Introduce a representative sample of the tomato extract into a vial and close it. Place the vial in a water bath at 50° for 30 minutes. (NOTE: The temperature should not exceed 60°). Stir the solution using a glass rod. Weigh accurately (to ±0.1 mg) 1.0 to 1.2 g of the sample into each of three 100-ml (V_A) volumetric flasks (samples 1, 2 and 3) and add 10 ml of BHT solution and 40 ml of dichloromethane to each flask. Homogenize the solutions using an ultrasonic bath, cool the solutions to room temperature and bring each to volume with dichloromethane and mix (Solutions C). Transfer 5 ml (V_B) of each Solution C to separate amber 50-ml (V_C) volumetric flasks. Bring each to volume with the Diluent solution and mix well (Solutions D).

Procedure

- Standardizing of the Lycopene standard solutions

Transfer 2.0 ml (V_D) of each of Solutions A and B into 100-ml (V_E) volumetric flasks and add 10 ml of ethanol and 10 ml of BHT solution. Bring the two solutions to volume with petroleum ether (Solutions E and F). Using a suitable UV/VIS spectrophotometer and 1-cm cell, determine the absorbances of these solutions at 472 nm using petroleum ether as a blank. Calculate the lycopene concentrations (C_{ST} mg/l) in Solutions A and B using the equation:

$$C_{ST}(\text{mg/l}) = \frac{A_{max} \times D \times 10000}{3450}$$

where

A_{max} is the absorbance of either Solution E or Solution F (corresponding to Solutions A and B, respectively) at 472 nm corrected for the blank;

D is the dilution factor V_E/V_D;

10000 is the scaling factor; and

3450 is the specific absorbance ($A^{1\%}_{1\,cm}$) of all-*trans*-lycopene in petroleum ether.

(NOTE: The lycopene concentrations of the standard Solutions A

and B should be redetermined prior to each separate analysis.)

- Chromatographic analysis

Inject Solutions A and B into the chromatograph. Record the peak areas. Inject the three sample solutions (Solutions D) and record the peak areas of lycopene (the retention time of all isomers of lycopene is approximately 5 to 7 min and that for β-carotene is 8 to 9 min).The peak area of lycopene for the sample solutions should be between 80 and 120% of the standards, otherwise dilute the Solution C with the Diluent solution to bring the lycopene concentration to the desired range or increase the sample weight.

- Calculation

Calculate the percentage of total lycopenes in sample 1 (TL_{1A}) as follows:

$$TL_{1A}(\%) = \frac{A_S \times C_{ST} \times V_A \times D}{A_{ST} \times W_S} \times 100$$

where

A_S is the peak area of the sample;
A_{ST} is the peak area of the standard Solutions A;
C_{ST} is the lycopene concentration in the standard solution A (mg/l);
V_A is the volume (l) used to dilute W_S to prepare solution C;
D is the dilution factor V_C/V_B; and
W_S is the sample weight (mg).

Repeat the calculation for the remaining two samples to obtain TL_{2A} and TL_{3A}.

Use the same procedure to calculate the percent of total lycopenes in the samples using the peak area of the standard Solutions B. Record the results as TL_{1B}, TL_{2B} and TL_{3B}. Calculate the mean percentage of total lycopenes in tomato extract.

TOTAL CAROTENOIDS

Using a volumetric pipette, transfer 2 ml (V_F) of Solution D (see above) to an amber 100-ml (V_G) volumetric flask. Add 10 ml of ethanol, bring to volume with petroleum ether and mix well. This is sample Solution G.

Using a suitable UV/VIS spectrophotometer and 1-cm sample cells with covers, scan the spectrum of Solution G from 550 to 300 nm, using petroleum ether as the reference blank and measure the absorbance at the absorbance maximum (approximately 472 nm). The absorbance should be between 0.2 and 0.8. Calculate the percentage of total carotenoids (as lycopene) in the sample using the following equation:

$$\text{Total carotenoids (as lycopene)}\ \% = \frac{A \times D}{W_S \times 3450} \times 100$$

where

A is the absorbance of Solution G at 472 nm, corrected for the blank;

3450 is the specific absorbance ($A^{1\%}_{1\ cm}$) of all-*trans*-lycopene in petroleum ether;
W_S is the weight of the sample (g); and
D is the dilution factor ($V_G x V_C / V_F x V_B$).