

## Lycopene

$C_{40}H_{56}$  536.88 [502-65-8].

Lycopene is a mixture of geometrical isomers of lycopene. It contains not less than 96.0 percent and not more than 101.0 percent of  $C_{40}H_{56}$ , calculated on the dried basis.

**Packaging and storage**— Preserve in tight, light-resistant containers, under inert gas, and store in a cool place.

**Labeling**— Label it to indicate whether the article is obtained from natural sources or is prepared synthetically. If obtained from natural sources, label it to indicate the natural source, including its Latin binomial.

**Identification**—

A: [Ultraviolet-Visible Absorption](#) [197U](#) —

Spectral range: 300 to 700 nm.

**Solution**— Prepare as directed below for the Test solution in the test for Content of lycopene.

Ratio:  $A_{476} / A_{508}$ , between 1.10 and 1.14.

B: The retention time of the major peak in the chromatogram of the Test solution corresponds to that in the chromatogram of the Standard solution, as obtained in the test for Content of all-E-lycopene, 5Z-lycopene, and related compounds.

[Loss on drying](#) [731](#) — Dry it in vacuum over phosphorus pentoxide at 40 °C for 4 hours: it loses not more than 0.2% of its weight.

[Residue on ignition](#) [281](#) : not more than 0.2%.

[Heavy metals, Method II](#) [231](#) : not more than 10 µg per g.

Content of all-E-lycopene, 5Z-lycopene, and related compounds—

Mobile phase— Prepare a filtered and degassed mixture of tert-butyl methyl ether, methanol, and tetrahydrofuran (784:665:74). Make adjustments if necessary.

Standard solution— Dissolve a suitable quantity of [USP Lycopene RS](#) in Mobile phase to obtain a solution containing about 2 mg per 100 mL.

Test solution— Transfer about 15 mg of Lycopene to a 25-mL volumetric flask, and dissolve in tetrahydrofuran containing 50 mg of butylated hydroxytoluene per L. Dilute with the same solvent

to volume. Pipet 2 mL into a 50-mL volumetric flask, and add 8 mL of tetrahydrofuran. Dilute with tert-butyl methyl ether to volume. Use this solution for injection.

**Chromatographic system**— The liquid chromatograph is equipped with a 472-nm detector, a 4.6-mm × 25-cm column that contains 5-µm packing L62, and a second column connected in series containing 3-µm packing L62. The flow rate is about 1.0 mL per minute. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 1.07 for 5Z-lycopene, and 1.0 for all-E-lycopene; the resolution, R, between all-E-lycopene and 5Z-lycopene is not less than 1.0; the tailing factor is not less than 0.8 and not more than 2.0; and the relative standard deviation for replicate injections for the all-E-lycopene is not more than 2.0%. [Note—New columns may require up to 30 injections before the system suitability requirements are met.]

**Procedure** —Inject about 10 µL of the Test solution into the chromatograph, record the chromatogram, and measure the peak area responses. Calculate the percentage of related compounds in the portion of Lycopene taken by the formula:

$$T(r_s / r_T)$$

in which T is the percentage of total lycopene isomers obtained in the test for Content of lycopene;  $r_s$  is the sum of the responses of all peaks except the peak for all-E-lycopene and the peak for 5Z-lycopene; and  $r_T$  is the total detected area. Not more than 9.0% of other related compounds calculated as lycopene are found. Calculate the percentage of the 5Z-lycopene isomer in the portion of Lycopene taken by the formula:

$$T(r_{5Z} / r_T)$$

in which  $r_{5Z}$  is the peak response for the 5Z-lycopene isomer, and the other terms are as described above. Not more than 23.0% of the 5Z-lycopene isomer is found. Calculate the percentage of all-E-lycopene taken by the formula:

$$T(r_E / r_T)$$

in which  $r_E$  is the peak response of the all-E-lycopene isomer, and the other terms are as described above. Not less than 70.0% of all-E-lycopene is found.

**Content of lycopene**—

Test stock solution— Transfer about 25 mg of Lycopene to a 100-mL volumetric flask, add about 25 mg of butylated hydroxytoluene and about 60 mL of methylene chloride, and sonicate to dissolve. Dilute with methylene chloride to volume.

Test solution— Transfer 2.0 mL of the Test stock solution to a 200-mL volumetric flask, and dilute with cyclohexane to volume.

Procedure— Determine the absorbance of the Test solution at the wavelength of maximum absorbance at about 476 nm, using cyclohexane as the blank. Calculate the percentage of C<sub>40</sub>H<sub>56</sub> in the portion of Lycopene taken by the formula:

$$1000A/331W$$

in which A is the absorbance of the Test solution; W is the weight, in g, of Lycopene taken to prepare the Test stock solution; and 331 is the absorptivity of the pure lycopene in cyclohexane.