Tomato Extract Containing Lycopene

Change to read:

» Tomato Extract Containing Lycopene is an ethyl acetate extract of the natural tomato lipids. It is produced from the pulp of ripe fruits of *Lycopersicon esculentum* Mill. (Fam. Solanaceae), •after removing the tomato water-soluble fraction.•(RB 1-Mar-2009) It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of lycopene ($C_{40}H_{56}$). It contains not less than 4.7 percent and not more than 12.0 percent of lycopene ($C_{40}H_{56}$), not less than 0.8 percent of the combined amount of phytofluene ($C_{40}H_{68}$) and phytoene ($C_{40}H_{64}$), not less than 0.2 percent of beta carotene ($C_{40}H_{56}$), and not less than 1.0 percent of tocopherols ($C_{28}H_{48}O_2$) on the anhydrous basis. Tocopherols may be added as antioxidants.

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

Labeling—Label it to state the content of lycopene in percentage and that the material should be heated to 50º and mixed before use. Label it to indicate the Latin binomial and the part of the plant from which the article is derived.

USP Reference standards ⟨11⟩**—***USP Lycopene RS. USP Tomato Extract Containing Lycopene RS.*

Clarity of solution—Warm the sample to 50° in a water bath. Mix well with a glass rod or a spatula. Accurately weigh about 1 g of the Extract directly into a 100-mL volumetric flask. Add 50 mL of methylene chloride, and sonicate the solution for 1 minute to completely dissolve the sample. Bring to room temperature, dilute with methylene chloride to volume, and mix well. The solution is clear: no deposit or turbidity is formed.

Change to read:

Identification—

A: PRESENCE OF LYCOPENE, PHYTOFLUENE, AND PHYTOENE—

Butylated hydroxytoluene stock solution, Diluting solution, Standard solution A, Standard solution B, and *Test solution*—Proceed as directed in the test for *Content of lycopene.*

Chromatographic system—Proceed as directed in the test for *Content of other carotenoids and tocopherols.* The retention times for lycopene peak, phytofluene peak, and phytoene peak in the chromatogram of the *Test solution* correspond to those in the chromatogram of the *System suitability solution,* as obtained in the test for *Content of other carotenoids and tocopherols.*

B: RATIO OF ALL-E-LYCOPENE AND 5Z-LYCOPENE—

Butylated hydroxytoluene stock solution—Proceed as directed in the test for *Content of lycopene.*

Mobile phase—Prepare a filtered and degassed solution of 0.05% diisopropylethylamine in *n*-hexane. Mix well, and sonicate for 3 to 4 minutes.

Test solution—Proceed as directed in the test for *Content of lycopene,* except to make the final dilution by transferring 5 mL to a 100-mL volumetric flask and diluting with *n*-hexane to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with two 4.0-mm × 25-cm columns that contain 5-µm packing L3 (300 Å pore size), connected in a series and kept at 22°, and a 472-nm detector. The flow rate is 0.5 mL per minute. The peak for all-*E*-lycopene elutes between 30 and 45 minutes; the relative retention time for all-*E*-lycopene is 1.00; and the relative retention time for 5*Z*-lycopene is in the range from 1.04 to 1.10.•(RB 1-Mar-2009)

Procedure—Inject about 10 µL of the *Test solution* into the chromatograph, record the chromatogram, measure the peak responses of the two major peaks, and calculate their area ratio by the formula:

$$r_{U1} / r_{U2}$$

in which r_{U2} is the peak response of all-*E*-lycopene; and r_{U1} is the peak response of 5*Z*-lycopene. The area ratio is not more than 0.10.

Viscosity ⟨911⟩**:** Equilibrate the Extract at 37° in a 30-mL glass vial. Determine the viscosity using a rotational viscosimeter equipped with a spindle (No. 6) having a cylinder 1.47 cm in diameter and 0.16 cm high attached to a shaft 0.32 cm in diameter, with a distance of 3.02 cm from the top of the cylinder to the lower tip of the shaft. The spindle is rotating at the appropriate speed and immersion depth to obtain a scale reading between 10% and 90% of full scale. Calculate the viscosity, in centipoises, by multiplying the scale reading by the constant for the spindle and speed used: the viscosity is not more than 5000 centipoises.

Microbial enumeration ⟨2021⟩**—**It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli,* and *Pseudomonas aeruginosa.* The total aerobic microbial count does not exceed 1000 cfu per g, and the total combined molds and yeasts count does not exceed 200 cfu per g.

Limit of aflatoxins ⟨561⟩**:** not more than 4 µg per kg of total aflatoxins B1, B2, G1, and G2; not more than 2 µg per kg of aflatoxin B1.

Water, *Method Ia* ⟨921⟩**:** not more than 0.8%.

Particle size distribution—Transfer 1 drop to a microscope slide, and spread evenly. Isopropanol may be used as a diluent, if necessary. Examine the slide under a microscope equipped with a calibrated ocular micrometer, using about 450 × magnification (see *Optical Microscopy* ⟨776⟩). Scan the slide, and note the size of the individual particles: not fewer than 98% of the particles are less than 20 µm in length when measured along the longest axis, not fewer than 60% of the particles are less than 5 µm, and not fewer than 40% of the particles are less than 2 µm.

Pesticide residues ⟨561⟩**:** meets the requirements.

Heavy metals, *Method II* ⟨231⟩**:** 10 µg per g.

Change to read:

Content of lycopene—

Butylated hydroxytoluene stock solution—Dissolve 2.5 g of butylated hydroxytoluene in methylene chloride to make 500 mL. [NOTE—This solution can be stored protected from light for up to 3 months.]

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, methylene chloride, and *n*-hexane (850 : 100 : 25 : 25). Add 0.05% of diisopropylethylamine, mix well, and sonicate for 3 to 4 minutes.

Diluting solution—Prepare a mixture of acetonitrile, methylene chloride, methanol, *n*-hexane, and butylated hydroxytoluene (600 : 150 : 150 : 100 : 0.5). Add 0.05% of diisopropylethylamine, mix well, and sonicate for 3 to 4 minutes.

Standard solution A—•Transfer an accurately weighed quantity of USP Lycopene RS, equivalent to approximately 5 mg of lycopene, into a 100-mL volumetric flask, add about 60 units of bacterial alkaline protease preparation or another suitable enzyme, and about 25 mg of butylated hydroxytoluene. Add 2.5 mL of dilute ammonium hydroxide (2 in 100) in water, mix, place in an ultrasonic bath at 50° for 10 minutes, rotate the flask occasionally to avoid having the material stick to the glass surface, and continue until the material is dispersed with no lumps. Add 5 mL of tetrahydrofuran, and shake until no colored precipitate remains. Add an-

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other portion of 2 mL of tetrahydrofuran, 40 mL of *Diluting solution*, and shake until the mixture is homogeneous. Complete to volume with *Diluting solution*, shake vigorously, and allow to stand, if necessary, until the solid has settled.•(RB 1-Mar-2009)

Determine the exact concentration of *Standard solution A* by employing the following method.

Standard solution B—Transfer 2.0 mL of *Standard solution A* to a 100-mL volumetric flask, add 10 mL of alcohol and 10 mL of *Butylated hydroxytoluene stock solution*, dilute with *n*-hexane to volume, and mix. •Prepare in triplicate.• (RB1-Mar-2009)

PROCEDURE—•Determine the absorbance of this solution at the maximum absorbance at about 472 nm using a mixture of alcohol, *Butylated hydroxytoluene stock solution*, and *n*-hexane (10 : 10 : 80) as the blank. Calculate the concentration of *Standard solution A*, in µg per mL, of lycopene, by the formula:•(RB 1-Mar-2009)

$$50{,}000A_x/345$$

in which A_x is the average of the absorbance of the three preparations of *Standard solution B*, and 345 is the absorptivity for pure lycopene in *n*-hexane at 472 nm.

Standard solution C—•Transfer an accurately weighed quantity of USP Tomato Extract Containing Lycopene RS, equivalent to about 6 mg of lycopene, to a 100-mL volumetric flask, and dissolve in 1 mL of *Butylated hydroxytoluene stock solution* and 9 mL of methylene chloride, using a sonicator. Dilute with *Diluting solution* to volume to obtain a solution having a known concentration of about 0.06 mg of lycopene per mL.• (RB 1-Mar-2009)

Test solution—Warm the sample of the Extract to 50° in a water bath. Mix well with a glass rod or a spatula. Accurately weigh a quantity of 1 to 1.2 g of the sample into a 100-mL volumetric flask. Add 10 mL of *Butylated hydroxytoluene stock solution* and 30 mL of methylene chloride, and sonicate the solution for 1 minute in order to dissolve the sample completely. Cool to room temperature, dilute with methylene chloride to volume, and mix well. Transfer 5.0 mL to a 50-mL volumetric flask, and dilute with *Diluting solution* to volume.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 472-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L7 maintained at a temperature of 39 ± 1°. The flow rate is about 0.7 mL per minute. Chromatograph *Standard solution A*, record the chromatograms, and measure the peak responses as directed for *Procedure:* the retention time for lycopene is about 6 minutes; and the relative standard deviation of the lycopene peak area for replicate injections is not more than 1.5%.

Procedure—Separately inject equal volumes (10 µL) of *Standard solution A* or *Standard solution C*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of the major lycopene peaks. Calculate the percentage of lycopene in the portion of Extract taken by the formula:

$$100(C/W)(r_U / r_S)$$

in which C is the concentration, •in µg per mL,• (RB 1-Mar-2009) of *Standard solution A* or *Standard solution C; W* is the weight, in mg, of Extract taken to prepare the *Test solution;* and r_U and r_S are the areas of the lycopene peak responses obtained from the *Test solution* and *Standard solution A* or *Standard solution C*, respectively.

Change to read:

Content of other carotenoids and tocopherols (phytofluene, phytoene, beta carotene, and tocopherols)—

Butylated hydroxytoluene stock solution, Diluting solution, Standard solution A, Standard solution B, Standard solution C, and *Test solution*—Proceed as directed in the test for *Content of lycopene.*

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, methanol, methylene chloride, and *n*-hexane (475 : 475 : 25 : 25). Add 0.05% of diisopropylethylamine, mix well, and sonicate for 3 to 4 minutes.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 4.6-mm × 25-cm column that contains 5-µm packing L1 maintained at a temperature of 39 ± 1º and a detector set at 472 nm for lycopene, at 450 nm for beta carotene, at 350 nm for phytofluene, and at 288 nm for phytoene and tocopherol. The flow rate is about 0.6 mL per minute.

Chromatograph *Standard solution C*, record the chromatograms, and measure the peak responses and the peak retention times as directed for *Procedure:* the chromatogram of *Standard solution C* is similar to the Reference Chromatogram provided with the USP Tomato Extract Containing Lycopene RS; the relative retention times in the chromatogram of *Standard solution C* are about 0.6 for the peaks of the tocopherol isomers, •1.0 for the peak of all-*E*-lycopene,•(RB 1-Mar-2009) 1.5 to 1.7 for the peaks of the beta carotene isomers, 1.6 to 1.8 for the peaks of the phytofluene isomers, and 1.8 to 2.2 for the phytoene peak; and the relative standard deviation for replicate injections for the peak of the lycopene isomers is not more than 2%.

Procedure—Separately inject equal volumes (10 µL) of *Standard solution A* and the *Test solution* into the chromatograph, and record the chromatograms. Identify the locus of the peaks for the lycopene isomers, beta carotene isomers, phytofluene isomers, and phytoene by comparison with the Reference Chromatogram provided with the corresponding lot of USP Tomato Extract Containing Lycopene RS. Measure the sum of the peak responses of the lycopene isomers at 472 nm in *Standard solution A.* •[NOTE—The lycopene isomers may be resolved in more than one peak in this chromatographic system.]•(RB 1-Mar-2009) In the *Test solution*, measure the sum of the peak responses of the beta carotene isomers at 450 nm, the sum of the peak responses of the phytofluene isomers at 350 nm, the response of the phytoene at 288 nm, and the sum of the peak responses of all tocopherols at 288 nm.

Determine the concentration of *Standard solution A* as directed in the test for *Content of lycopene.*

Calculate the percentage of beta carotene in the portion of Extract taken by the formula:

$$100(C/W)(r_{U1} / r_S)(345/259.2)$$

•in which C is the concentration, in µg per mL, of *Standard solution A* ; W is the weight,• (RB 1-Mar-2009) in mg, of Extract taken to prepare the *Test solution;* r_{U1} is the sum of the peak responses for the beta carotene isomers at 450 nm obtained from the *Test solution;* r_S is the sum of the peak responses for the lycopene isomers at 472 nm obtained from *Standard solution A;* and 345 and 259.2 are the absorptivities for pure lycopene and for pure beta carotene, respectively.

Calculate the percentage of phytofluene in the portion of Extract taken by the formula:

$$100(C/W)(r_{U2} / r_S)(345/135)$$

•in which C is the concentration, in µg per mL, of *Standard solution A*; W is the weight,• (RB 1-Mar-2009) in mg, of Extract taken to prepare the *Test solution;* r_{U2} is the sum of the peak responses for phytofluene isomers at 350 nm obtained from the *Test solution;* r_S is the sum of the peak responses for the lycopene isomers at 472 nm obtained from *Standard solution A*••(RB 1-Mar-2009); and 345 and 135 are the absorptivities for pure lycopene and for pure phytofluene, respectively.

Calculate the percentage of phytoene in the portion of Extract taken by the formula:

$$100(C/W)(r_{U3} / r_S)(345/125)$$

•in which C is the concentration, in µg per mL, of *Standard solution A*; W is the weight,• (RB 1-Mar-2009) in mg, of Extract taken to pre-

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pare the *Test solution;* r_{U3} is the area of the phytoene peak response at 288 nm obtained from the *Test solution;* r_S is the sum of the peak responses for the lycopene isomers at 472 nm obtained from *Standard solution A;* and 345 and 125 are the absorptivities for pure lycopene and for pure phytoene, respectively.

Calculate the percentage of tocopherols in the portion of Extract taken to prepare the *Test solution* by the formula:

$$100(C/W)(r_{U4} / r_S)(345/8.5)$$

•in which C is the concentration, in µg per mL, of *Standard solution A*; W is the weight,• (RB 1-Mar-2009) in mg, of Extract taken to prepare the *Test solution;* r_{U4} is the sum of the peak responses for all the tocopherol peaks at 288 nm obtained from the *Test solution;* r_S is sum of the peak responses for the lycopene isomers at 472 nm obtained from *Standard solution A;* 345 is the absorptivity for pure lycopene; and 8.5 is the average absorptivity for tocopherols.

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