

Powdered Decaffeinated Green Tea Extract

Powdered Decaffeinated Green Tea Extract is prepared from the young, unfermented leaf and leaf buds of *Camellia sinensis* (L.) Kuntze (Fam. Theaceae), also known as *Thea sinensis* L., using suitable solvents such as alcohol, methanol, acetone, or water or mixtures of these solvents; the caffeine has been removed. The ratio of the starting crude plant material to Powdered Extract is between 6:1 and 10:1. It contains not less than 60.0 percent of polyphenols, calculated as (-)-epigallocatechin-3-O-gallate, not less than 40.0 percent of (-)-epigallocatechin-3-O-gallate, and not more than 0.1 percent of caffeine, calculated on the anhydrous basis.

Packaging and storage— Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.

Labeling— The label states the Latin binomial and, following the official name, the part of the plant contained in the article. It meets other labeling requirements under [Botanical Extracts](#)

[565](#) .

USP Reference standards [11](#) —

[USP Caffeine RS](#). USP (-)-Epigallocatechin-3-O-gallate.

[USP Powdered Decaffeinated Green Tea Extract RS](#).

Identification—

A: [Thin-Layer Chromatographic Identification Test](#) [201](#) —

Standard solution— Dissolve about 40 mg of [USP Powdered Decaffeinated Green Tea Extract RS](#) in 10 mL of a mixture of alcohol and water (8:2) by sonication for 10 minutes, and centrifuge.

Use the clear supernatant. [Note—Prepare fresh. Store below -20°C, if storage is needed.]

Test solution— Proceed as directed for Standard solution, except to use Powdered Extract.

Adsorbent— Use a chromatographic silica gel mixture with an average particle size of 5 µm.

Application volume: 1 µL.

Developing solvent system— Use a mixture of toluene, acetone, and formic acid (9:9:2).

Immersion reagent— Dissolve 140 mg of fast blue B salt in 10 mL of water, add 140 mL of methanol and 50 mL of dichloromethane, and mix. [Note—Prepare weekly and store at 4°C in the dark.]

Procedure— [Note—Use an unsaturated chamber, and condition the plate to a relative humidity of about 30% using a suitable device.] Develop the chromatograms using Developing solvent system until the solvent front has moved about three-fourths of the plate, dry the plate at 100 °C, dip in the Immersion reagent, dry, and immediately examine the plate under visible light [Note—The chromatogram is stable up to 30 minutes; afterward, the plate's background darkens significantly.]: the chromatogram of the Test solution exhibits main bands similar in position and color to the main bands in the chromatogram of the Standard solution. The chromatogram of the Test solution exhibits four main brownish-orange bands with R_F values of approximately 0.38, 0.48, 0.52, and 0.62 corresponding to (-)-epigallocatechin-3-O-gallate, (-)-epigallocatechin, (-)-epicatechin-3-O-gallate, and (-)-epicatechin, respectively. The most intense band is the one for (-)-epigallocatechin-3-O-gallate. The least intense band is the one for (-)-epicatechin.

B: The retention times of the peaks for (-)-epigallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin-3-O-gallate, (-)-gallocatechin-3-O-gallate, (-)-epigallocatechin-3-O-(3-O-methyl)-gallate, and (-)-epicatechin-3-O-gallate in the chromatogram of the Test solution correspond to those in the chromatogram of Standard solution 2, as obtained in the test for Content of polyphenols.

Microbial enumeration [2021](#) — The total aerobic microbial count does not exceed 10⁴ cfu per g. The total combined yeasts and molds count does not exceed 10³ cfu per g.

Absence of specified microorganisms [2022](#) — It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

Water, [Method Ia](#) [921](#) : not more than 6.0%, determined on 0.5 g.

Residue on ignition [281](#) : not more than 0.5%, determined on 1.0 g.

Heavy metals, [Method II](#) [231](#) : not more than 20 µg per g.

Pesticide residues [561](#) : meets the requirements.

Limit of gallic acid—

Solution A, Solution B, Mobile phase, and Chromatographic system—Proceed as directed in the test for Content of polyphenols.

Standard solution— Dissolve an accurately weighed quantity of gallic acid in Solution A to obtain a solution having a known concentration of about 0.2 mg per mL.

Test solution— Transfer about 500 mg of Powdered Extract, accurately weighed, to a 25-mL volumetric flask, dissolve, dilute with Solution A to volume, mix, and centrifuge.

Procedure— Separately inject equal volumes (about 15 μ L) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas of the gallic acid peaks. Separately calculate the percentages of gallic acid in the portion of Powdered Extract taken using the formula:

$$2500(C / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of gallic acid in the Standard solution; W is the weight, in mg, of Powdered Extract taken to prepare the Test solution; and r_U and r_S are the peak responses obtained for gallic acid in the Test solution and the Standard solution, respectively: not more than 1.0% is found.

Limit of caffeine—

Solution A— Prepare a filtered and degassed mixture of water, methanol, tetrahydrofuran, and phosphoric acid 85% (936.5:50:10:3.5).

Solution B— Prepare a filtered and degassed mixture of acetonitrile, methanol, and phosphoric acid 85% (946.5:50:3.5).

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard solution— Dissolve an accurately weighed quantity of [USP Caffeine RS](#) in methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

Test solution— Transfer about 10 mg of Powdered Extract, accurately weighed, to a 10-mL volumetric flask, add 5 mL of methanol, dissolve, dilute with methanol to volume, mix, and centrifuge.

Chromatographic system— The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm \times 25-cm column that contains 5- μ m packing L60.* The flow rate is about 1.0 mL per

minute. The column temperature is maintained at 25 ± 1 . The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–30	100	0	isocratic
30–35	100→0	0→100	linear gradient
35–40	0	100	isocratic
40–45	0→100	100→0	linear gradient
45–55	100	0	isocratic

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative standard deviation determined from the caffeine peak for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 15 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas of the caffeine peaks. Separately calculate the percentages of caffeine in the portion of Powdered Extract taken using the formula:

$$1000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of [USP Caffeine RS](#) in the Standard solution; W is the weight, in mg, of Powdered Extract taken to prepare the Test solution; and r_U and r_S are the peak responses obtained for caffeine in the Test solution and the Standard solution, respectively: not more than 0.1% is found.

Content of polyphenols—

Solution A— Prepare a filtered and degassed mixture of water, methanol, and phosphoric acid 85% (946.5:50:3.5).

Solution B— Prepare a filtered and degassed mixture of acetonitrile and methanol (95:5).

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard solution 1— Dissolve an accurately weighed quantity of USP

(-)-Epigallocatechin-3-O-gallate RS in Solution A to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, and mix.

Standard solution 2— Transfer about 20 mg of [USP Powdered Decaffeinated Green Tea Extract RS](#), accurately weighed, to a 10-mL volumetric flask, add 5 mL of Solution A, dissolve, dilute with the same solvent to volume, and mix. Transfer 2.0 mL of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, mix, and centrifuge.

Test solution— Proceed as directed for Standard solution 2, except to use Powdered Extract.

Chromatographic system— The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 0.8 mL per minute. The column temperature is maintained at 25 ± 1°C. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–20	94	6	isocratic
20–50	94→78	6→22	linear gradient
50–70	78→38	22→62	linear gradient
70–75	38→94	62→6	isocratic
75–90	94	6	isocratic

Chromatograph Standard solution 1, and record the peak responses as directed for Procedure: the tailing factor for the (-)-epigallocatechin-3-O-gallate peak is not less than 0.8 and not more than 2.0; and the relative standard deviation determined from the (-)-epigallocatechin-3-O-gallate peak for replicate injections is not more than 2.0%. Chromatograph Standard solution 2, and record the peak responses as directed for Procedure: the chromatogram obtained is similar to the Reference chromatogram provided with the lot of [USP Powdered Decaffeinated Green Tea Extract RS](#) being used; and the resolution, R, between the (-)-epigallocatechin-3-O-gallate peak and the preceding peak is not less than 1.

Procedure— Separately inject equal volumes (about 15 µL) of Standard solution 1, Standard solution 2, and the Test solution into the chromatograph; record the chromatograms; and measure the areas of the analyte peaks. Using the chromatogram of Standard solution 2 and the Reference chromatogram provided with the lot of [USP Powdered Decaffeinated Green Tea Extract RS](#), identify the retention times of the peaks corresponding to the different polyphenols. The approximate relative retention times of the polyphenols are provided in the following table:

Analyte	Relative Retention Time
(-)-Epigallocatechin	0.56
(+)-Catechin	0.68
(-)-Epicatechin	0.98
(-)-Epigallocatechin-3-O-gallate	1.00
(-)-Gallocatechin-3-O-gallate	1.09
(-)-Epigallocatechin-3-O-(3'-O-methyl)-gallate	1.19
(-)-Epicatechin-3-O-gallate	1.27

Separately calculate the percentages of (-)-epigallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin-3-O-gallate, (-)-gallocatechin-3-O-gallate, (-)-epigallocatechin-3-O-(3'-O-methyl)-gallate, and (-)-epicatechin-3-O-gallate as (-)-epigallocatechin-3-O-gallate in the portion of the Powdered Extract taken using the formula:

$$5000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP (-)-Epigallocatechin-3-O-gallate RS in Standard solution 1; W is the weight, in mg, of Powdered Extract taken to prepare the Test solution; r_U is the peak response obtained for each of the polyphenols in the Test solution; and r_S is the peak response obtained for (-)-epigallocatechin-3-O-gallate in Standard solution 1: not less than 40.0% of (-)-epigallocatechin-3-O-gallate is found. Add the percentages calculated for the individual analytes: not less than 60.0% of polyphenols, calculated as (-)-epigallocatechin-3-O-gallate, is found.

Other requirements— It meets the requirements of the test for Residual Solvents under [Botanical](#)

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