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Powdered Decaffeinated Green Tea Extract

DEFINITION

Powdered Decaffeinated Green Tea Extract is prepared from young unfermented leaves and leaf buds of *Camellia sinensis* (L.) Kuntze (Family Theaceae) [syn. *Thea sinensis* L.] using suitable solvents such as alcohol, methanol, acetone, water, or mixtures of these solvents; the caffeine has been removed. The ratio of the starting crude plant material to Powdered Decaffeinated Green Tea Extract is 6:1–10:1. It contains NLT 60.0% of polyphenols, calculated as (–)-epigallocatechin-3-O-gallate, NLT 40.0% of (–)-epigallocatechin-3-O-gallate, and NMT 0.1% of caffeine, all calculated on the anhydrous basis.

IDENTIFICATION

• **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).**

Standard solution: 4 mg/mL of [USP Powdered Decaffeinated Green Tea Extract RS](#) in [alcohol](#) and [water](#) (4:1), sonicated for 10 min, and centrifuged. Use the clear supernatant. [NOTE—Prepare fresh. Store below –20°, if storage is needed.]

Sample solution: 4 mg/mL of Powdered Decaffeinated Green Tea Extract in [alcohol](#) and [water](#) (4:1), sonicated for 10 min, and centrifuged. Use the clear supernatant.

Chromatographic system

Application volume: 1 µL

Developing solvent system: [Toluene](#), [acetone](#), and [formic acid](#) (9:9:2)

Immersion reagent: Dissolve 140 mg of [fast blue B salt](#) in 10 mL of [water](#), and add 140 mL of [methanol](#) and 50 mL of [dichloromethane](#).

[NOTE—Prepare weekly, and store at 4° in the dark.]

Analysis

Samples: *Standard solution* and *Sample solution*

Use an unsaturated chamber. Develop the chromatograms, dry the plate at 100°, treat with the *Immersion reagent*, dry in a current of cold air, and immediately examine under white light. [NOTE—The chromatogram is stable up to 30 min; afterward, the plate's background darkens significantly.]

System suitability: Under white light, the derivatized chromatogram of the *Standard solution* exhibits, in the middle third of the plate, four prominent brownish-orange bands corresponding, in the order of increasing R_f , to (–)-epigallocatechin-3-O-gallate, (–)-epigallocatechin, (–)-epicatechin-3-O-gallate, and (–)-epicatechin, respectively. The most intense band is that of (–)-epigallocatechin-3-O-gallate.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits major bands similar in position and color to the major bands of the *Standard solution*.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Polyphenols*.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits 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 at retention times corresponding to those of *Standard solution B*.

COMPOSITION

• **CONTENT OF POLYPHENOLS**

Solution A: [Methanol](#), 85% [phosphoric acid](#), and [water](#) (50:3.5:946.5)

Solution B: [Acetonitrile](#) and [methanol](#) (95:5)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
20	94	6
50	78	

Time (min)	Solution A (%)	Solution B (%)
70	38	62
75	94	6
90	94	6

Standard solution A: 0.1 mg/mL of [USP \(-\)-Epigallocatechin-3-O-gallate RS](#) in *Solution A*

Standard solution B: 0.4 mg/mL of [USP Powdered Decaffeinated Green Tea Extract RS](#) in *Solution A*. Mix and centrifuge.

Sample solution: 0.4 mg/mL of Powdered Decaffeinated Green Tea Extract in *Solution A*. Mix and centrifuge.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 25°

Flow rate: 0.8 mL/min

Injection volume: 15 μL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1 between the (-)-epigallocatechin-3-O-gallate peak and the preceding peak, *Standard solution B*

Tailing factor: 0.8–2.0 for the (-)-epigallocatechin-3-O-gallate peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the (-)-epigallocatechin-3-O-gallate peak in replicate injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of [USP Powdered Decaffeinated Green Tea Extract RS](#) being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Record the chromatograms, and measure the areas of the analyte peaks. Using the chromatogram of *Standard solution B* 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 [Table 2](#).

Table 2

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 Powdered Decaffeinated Green Tea Extract taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

r_U = peak area of each of the polyphenols in the *Sample solution*

r_S = peak area of (-)-epigallocatechin-3-O-gallate in *Standard solution A*