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^Guarana Seed Powder

DEFINITION

Guarana Seed Powder consists of the dried seeds of *Paullinia cupana* Kunth [syn. *P. cupana* var. *sorbilis* (Mart.) Ducke] (Family Sapindaceae) reduced to a fine or very fine powder. It contains NLT 3.5% of caffeine ($C_8H_{10}N_4O_2$) on the dried basis and NLT 1.5% of total flavonoids calculated as the sum of procyanidin B₁ ($C_{30}H_{26}O_{12}$), catechin ($C_{15}H_{14}O_6$), procyanidin B₂ ($C_{30}H_{26}O_{12}$), and epicatechin ($C_{15}H_{14}O_6$), on the dried basis.

IDENTIFICATION

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

Standard solution A: 1.0 mg/mL of [USP Caffeine RS](#) in [methanol](#)

Standard solution B: 0.5 mg/mL each of [USP \(-\)-Epicatechin RS](#) and [USP Procyanidin B₂ RS](#) in [methanol](#)

Sample solution: 1 g of Guarana Seed Powder in 10 mL of [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Chromatographic system

Adsorbent: [Silica gel, precoated plates, with fluorescence indicator F₂₅₄](#), for HPTLC, size 20 cm × 10 cm

Application volume: 4 µL, as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

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

Developing distance: 6 cm from the application line

Derivatization reagent: 170 mL of ice-cooled methanol mixed with 20 mL of glacial acetic acid, 10 mL of sulfuric acid, and 1 mL of [p-anisaldehyde](#). [NOTE—Use the spray derivatization technique with 5 mL of reagent.]

Analysis

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

Apply the *Samples* as bands and dry in air. Develop in an unsaturated chamber, remove the plate from the chamber, and dry in air.

Examine under UV light at 254 nm. Treat the plate with *Derivatization reagent*, heat for 3 min at 100°, and examine under white light.

System suitability

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

Suitability requirements: Prior to derivatization, under UV light at 254 nm, *Standard solution A* exhibits a quenching band in the upper-third section due to caffeine. After derivatization, under white light, *Standard solution B* exhibits a reddish-brown band in the middle-third section due to epicatechin and a light-brown band in the lower-third section corresponding to procyanidin B₂.

Acceptance criteria: Prior to derivatization, under UV light at 254 nm, the *Sample solution* exhibits a quenching band corresponding in *R_f* and color to caffeine in *Standard solution A*. After derivatization, under white light, the *Sample solution* exhibits brown bands corresponding in *R_f* and color to epicatechin (in coelution with catechin) and procyanidin B₂ in *Standard solution B*. The *Sample solution* also exhibits two additional light-brown bands between epicatechin and procyanidin B₂, a reddish-brown band below procyanidin B₂, and two gray bands close to the application position.

• B. HPLC

Analysis: Proceed as directed in the test for *Content of Caffeine and Flavonoids*.

Acceptance criteria: The *Sample solution* exhibits a principal dominant peak with a retention time corresponding to caffeine in *Standard solution A* and peaks due to catechin, procyanidin B₂, and epicatechin in *Standard solution B*. Minor peaks due to theobromine, theophylline, procyanidin B₁, and an unknown B-type dimeric procyanidin, as well as an unknown peak eluting just before epicatechin, and another unknown peak eluting at a relative retention time (RRT) of about 1.1 with respect to epicatechin, are observed at the retention times corresponding to the same constituents in *Standard solution C*. No other peak in the chromatogram of the *Sample solution* is more intense than the peak for procyanidin B₁. The area ratio of catechin to epicatechin is about 1:1.

COMPOSITION

• CONTENT OF CAFFEINE AND FLAVONOIDS

[NOTE—The *Standard solutions* and the *Sample solution* are stable for 24 h at room temperature.]

Solution A: 1.36 g/L of [monobasic potassium phosphate](#); adjusted with 3.0% [phosphoric acid](#) to a pH of 3.1



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Solution B: [Acetonitrile](#) and [methanol](#) (70:30)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	90	10
20	85	15
30	85	15
35	70	30
40	70	30
45	90	10
50	90	10

Solvent: [Methanol](#) and [water](#) (80:20)

Standard solution A: 0.50 mg/mL of [USP Caffeine RS](#) in *Solvent*

Standard solution B: 0.20 mg/mL of [USP \(-\)-Epicatechin RS](#), 0.3 mg/mL of [USP \(+\)-Catechin RS](#), and 0.10 mg/mL of [USP Procyanidin B₂ RS](#) in [water](#) and [methanol](#) (80:20). Sonicate to dissolve.

Standard solution C: 25 mg/mL of [USP Guarana Seed Dry Extract RS](#) in [water](#) and [methanol](#) (80:20). Sonicate for 10 min. Before injection, pass through a suitable membrane filter of 0.22-µm pore size.

Sample solution: Weigh about 500 mg of Guarana Seed Powder into a 100-mL round-bottom flask. Add 60 mL of *Solvent* and keep in a water bath under reflux at 70° for 30 min. Cool and transfer the extract along with residue into a 100-mL volumetric flask. Rinse the round-bottom flask with *Solvent*, transfer to the 100-mL volumetric flask, and add *Solvent* to volume. Before injection, pass through a suitable membrane filter of 0.22-µm pore size and discard the first portion of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 250-mm; 5-µm packing [L1](#)

Column temperature: 25°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

Suitability requirements

Resolution: NLT 3.0 between caffeine and catechin, *Standard solution C*

Tailing factor: NMT 2.0 for caffeine, *Standard solution A*; NMT 1.5 for catechin, epicatechin, and procyanidin B₂, *Standard solution B*

Relative standard deviation: NMT 2.0% for caffeine in repeated injections, *Standard solution A*; NMT 5.0% for catechin, epicatechin, and procyanidin B₂ in repeated injections, *Standard solution B*

Chromatogram similarity: The chromatogram of *Standard solution C* is similar to the reference chromatogram provided with the lot of [USP Guarana Seed Dry Extract RS](#) being used.

Analysis

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

Inject the *Samples* and register the chromatogram between 0.3 (RRT of theobromine) and 1.4 (RRT of epicatechin). Using the chromatogram of *Standard solution C* and the reference chromatogram provided with the lot of [USP Guarana Seed Dry Extract RS](#) being used, identify the retention times of the relevant peaks in the *Sample solution*.

Calculate the percentage of caffeine in the portion of Guarana Seed Powder taken:

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

r_U = peak area of caffeine from the *Sample solution*