Development of USP Guarana Seed Monograph Family

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The United States Pharmacopeia (USP) has recently published new Dietary Supplement monographs for Guarana Seed, Guarana Seed Powder and Guarana Seed Dry Extract in the Pharmacopeial Forum (PF) *44(2)*- March-April, 2018 (http://www.usppf.com/pf/pub/index.html)
USP seeks the input and comments from stakeholders for the tests, specifications and acceptance criteria proposed in these monographs.

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Introduction

Paullinia cupana Kunth [syn. *P. cupana* var. *sorbilis* (Mart.) Ducke] (Family *Sapindaceae*) seed is highly valued because of the high caffeine content, which could be up to 6%. Besides its use in soft and energy drinks, approximately 30% of the guarana seed production serves as a raw material for the Dietary Supplement industry. The widespread use, high demand and scarce sources of Guarana Seed, prompts the need for the creation of global public standards to assure the correct identity, composition and purity of this ingredient and derived extracts. Considering this, the United States Pharmacopeia (USP) has recently published new Dietary Supplement monographs for Guarana Seed, Guarana Seed Powder and Guarana Seed Dry Extract in the Pharmacopeial Forum (PF) *44(2)* March-April, 2018:

- The proposal includes tests for identification and composition targeting methylxanthines (caffeine, theobromine, and theophylline) and flavonoids (catechin, epicatechin, epigallocatechin, procyanidin B1 and procyanidin B2) as marker compounds.
- New HPTLC and HPLC-DAD methods were developed and validated for the analysis of both classes of compounds in a single chromatographic run
- In addition to USP Caffeine RS and USP Catechin RS, two new reference standards (USP Epicatechin RS and USP Procyanidin B2 RS) are proposed

To our knowledge, this is the first attempt to include the full HPLC profile of methylxanthines and flavonoids for the creation of a global public standard for Guarana seed. USP seeks the input and comments from stakeholders for the tests, specifications and acceptance criteria proposed in these monographs

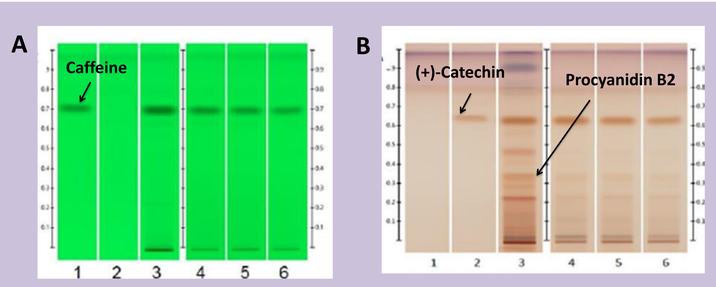
Identification

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 B_2 RS in methanol
Sample solution: 1 g of Guarana Seed (finely powdered), Guarana Seed Powder or Guarana Seed Dry Extract in 10 mL of methanol. Sonicate for 10 min, centrifuge, and use the supernatant.
Chromatographic system
Adsorbent: Silica gel, precoated plates, with fluorescence indicator F_{254}, 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: 70 mm from the lower edge of the plate
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
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 B_2.

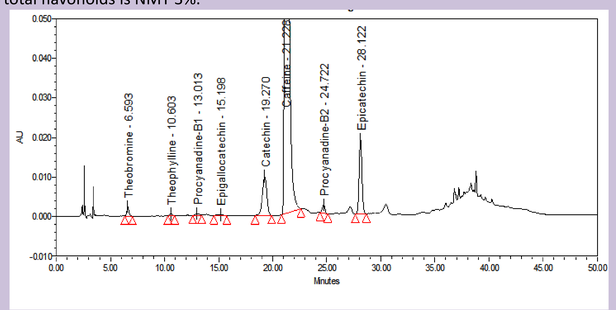
Acceptance criteria
- Prior to derivatization, under UV light at 254 nm, the *Sample solution* exhibits a quenching band corresponding in R_F and color to caffeine in *Standard solution A*.
- After derivatization, under white light, the *Sample solution* exhibits brown bands corresponding in R_F and color to epicatechin (in coelution with catechin) and procyanidin B_2 in *Standard solution B*.
- The *Sample solution* also exhibits two additional light-brown bands between epicatechin and procyanidin B_2, a reddish-brown band below procyanidin B_2, and two gray bands close to the application position.



HPLTC images: ***(A)*** *Prior to derivatization under UV 254 nm;* ***(B)*** *After derivatization with p-anisaldehyde under white light . Tracks* ***1:*** *Caffeine (1 mg/mL);* ***2****: (+)-Catechin (0.2 mg/mL);* ***3****: Guarana seed (100 mg/mL);* ***4, 5, 6****: Guarana seed dry extract (25 mg/mL)*

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 B_2, and epicatechin in *Standard solution B*. Minor peaks due to theobromine, theophylline, procyanidin B_1, and epigallocatechin, as well as one 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 B_1. The area ratio of catechin to epicatechin is about 1:1.
For *Guarana Seed Dry Extract*, the content ratio of catechin to epicatechin is about 2:1. The content ratio of caffeine to total flavonoids is NMT 3%.



HPLC Chromatogram at 275 nm of Caffeine and Flavonoids in Guarana Seed Powder

Composition

CONTENT OF CAFFEINE AND FLAVONOIDS

Solution A: 1.36 g/L of monobasic potassium phosphate; adjusted with 3.0% phosphoric acid to a pH of 3.1
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 B_2 RS in *Solvent*
Standard solution C: 25 mg/mL of USP Guarana Seed Dry Extract RS in water and methanol (80:20). Sonicate for 30 min. Before injection, pass through a suitable membrane filter of 0.22-µm pore size.
Sample solution: Weigh about 500 mg of *Guarana Seed (finely powdered) or 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. For *Guarana Seed Dry Extract*, prepare 5 mg/mL in water and methanol (80:20). Sonicate for 30 min.

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 1.5 for caffeine, *Standard solution A*; NMT 1.5 for catechin, epicatechin, and procyanidin B_2, *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 B_2 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 0.8 (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 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
r_S = peak area of caffeine from Standard solution A
C_S = concentration of USP Caffeine RS in Standard solution A (mg/mL)
V = volume of the Sample solution (mL)
W = weight of Guarana Seed used to prepare the Sample solution (mg)

Calculate the percentages of procyanidin B_1, epigallocatechin, catechin, procyanidin B_2, and epicatechin in the portion of Guarana Seed taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 1/F \times 100$$

r_U = peak area of the relevant analyte from the Sample solution
r_S = peak area of catechin, procyanidin B_2, or epicatechin from Standard solution B
C_S = concentration of USP (+)-Catechin RS, USP Procyanidin B_2 RS, or USP (−)-Epicatechin RS in Standard solution B (mg/mL)
V = volume of the Sample solution (mL)
W = weight of Guarana Seed used to prepare the Sample solution (mg)
F = relative response factor for the relevant analyte (For epigallocatechin use F=0.35 based on epicatechin, and for procyanidin B_1 use F=0.89 based on procyanidin B_2.)

Acceptance criteria:
Guarana Seed and Guarana Seed Powder. *Caffeine:* NLT 3.5% on the dried basis. *Flavonoids:* NLT 1.5% on the dried basis
Guarana Seed Dry Extract. *Caffeine*: 90.0%–110.0% of the labeled amount of caffeine calculated on the dried basis. The ratio of caffeine to total flavonoids is NMT 3%.

FOR CONTAMINANTS, SPECIFIC TESTS AND ADDITIONAL REQUIREMENTS. Please see *PF 44(2):* http://www.usppf.com/pf/pub/index.html