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Powdered Stinging Nettle Extract

DEFINITION

Powdered Stinging Nettle Extract is prepared from comminuted Stinging Nettle with 60% alcohol or other suitable solvents. It contains NLT 5.0% of total amino acids, NLT 0.1% of β -sitosterol ($C_{29}H_{50}O$), and NLT 30 $\mu\text{g/g}$ of scopoletin ($C_{10}H_8O_4$). The ratio of the starting crude plant material to Powdered Extract is 10:1.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution: 0.05 mg/mL of [USP Scopoletin RS](#) and 0.5 mg/mL of USP β -Sitosterol RS in methanol

Sample solution: Dissolve 0.6 g of Powdered Extract, in a mixture of toluene, ethyl acetate, and methanol (7:2:1). Filter, and dry under reduced pressure at a temperature below 40°. Dissolve the residue in 2.0 mL of the mixture containing toluene, ethyl acetate, and methanol.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.50-mm layer of chromatographic silica gel mixture

Application volume: 20 μL for the *Sample solution*; 10 μL for the *Standard solution*

Developing solvent system: Diethyl ether and methanol (9:1)

Spray reagent: 85% phosphoric acid, 10% vanillin in 96% ethanol, and water (4.5:1:4.5)

Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Examine the plates under UV light at 365 nm. Spray the plate with 10 mL of *Spray reagent*, heat between 100° and 105° for 10 min, and examine under daylight.

Acceptance criteria: The chromatogram of the *Sample solution* exhibits a violet-red zone corresponding to β -sitosterol at the same R_f value as β -sitosterol from the *Standard solution*, weakly visible zones above and below β -sitosterol, and a violet-red zone corresponding to β -sitosterolglucoside.

• B. GAS CHROMATOGRAPHY IDENTIFICATION TEST

Analysis: Proceed as directed in the test for *Content of β -Sitosterol*.

Acceptance criteria: The retention time of β -sitosterol of the *Sample solution* corresponds to that of the *Standard solution*.

• C. HPLC IDENTIFICATION TEST

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

Acceptance criteria: The retention time of scopoletin of the *Sample solution* corresponds to that of the *Standard solution*.

COMPOSITION

• CONTENT OF β -SITOSTEROL

Derivatizing reagent: A solution containing equal volumes (1:1:1) of BSTFA [*N,O*-bis(trimethylsilyl)tri fluoroacetamide], anhydrous pyridine, and a mixture of BSA [*N,O*-(trimethylsilyl)acetamide], TMSI (*N*-trimethylsilylimidazole), and TMCS (trimethylchlorosilane) (3:3:2)

Internal standard solution: 10 mg/mL of cholesterol in chloroform

Standard solution: Dissolve 50.0 mg of USP β -Sitosterol RS in 2 mL of chloroform, add 1.0 mL of *Internal standard solution*, and dilute to 5 mL with chloroform. Dry 0.5 mL under reduced pressure, and add 1 mL of *Derivatizing reagent*.

Sample solution: Transfer 20.0 g of Powdered Extract to a Soxhlet apparatus, treat with chloroform, and extract for 6 h. The volume of chloroform used is at least twice the volume of the thimble with an appropriately sized flask. Dry the solvent under reduced pressure, add 1.0 mL of *Internal standard solution*, and dilute with chloroform to 10 mL. Transfer 0.5 mL of this solution to a 10-mL round-bottom flask, dry the solvent under reduced pressure, and add 0.5 mL of *Derivatizing reagent*.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.20-mm × 25-m fused-silica capillary; 0.35-µm film of phase G2 coating

Temperature

Injector: 325°

Detector: 325°

Column: 300°, for 60 min

Carrier gas: Helium

Flow rate: 0.5 mL/min

Injection size: 1 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for each sterol peak

Relative standard deviation: NMT 5.0% determined from each sterol peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of β-sitosterol in the portion of Powdered Extract taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = peak response ratio of β-sitosterol to the internal standard from the *Sample solution*

R_S = peak response ratio of β-sitosterol to the internal standard from the *Standard solution*

W_S = weight of USP β-Sitosterol RS used to prepare the *Standard solution* (mg)

W_U = weight of Powdered Extract in the *Sample solution* (mg)

Acceptance criteria: NLT 0.1%

• CONTENT OF SCOPOLETIN

Solution A: Water

Solution B: Methanol

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
2	60	40
8	60	40
10	0	100
15	0	100
20	75	25
30	75	25

Standard solution: 0.02 µg/mL of [USP Scopoletin RS](#) in methanol

Sample stock solution: 8 mg/mL of Powdered Extract in methanol. Place in an ultrasonic bath for 25 min, and centrifuge.

Sample solution: *Sample stock solution* diluted with methanol 1 in 20

Chromatographic system

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

Mode: LC

Detector: Fluorescence; set at an excitation wavelength of 366 nm and an emission wavelength of 420 nm