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Pygeum Extract

DEFINITION

Pygeum Extract is prepared from pulverized Pygeum, using suitable solvents. It contains NLT 90% and NMT 110% of the labeled amount of docosyl ferulate and NLT 90% and NMT 110% of the labeled amount of total sterols as β -sitosterol, calculated on the dried basis.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution A: 15 mg/mL of [USP Pygeum Extract RS](#) in chloroform

Standard solution B: 2 mg/mL of USP β -Sitosterol RS in chloroform

Sample solution: Dissolve 150 mg of Extract in 10 mL of chloroform.

Chromatographic system

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

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 μ L

Developing solvent system: Methylene chloride in a saturated chamber

Spray reagent: Sulfuric acid and water (1:1)

Analysis

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

Develop the chromatograms to a length of NLT 15 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent*, and heat the plate at 100° for 10 min. Examine the plate under white light.

Acceptance criteria: The chromatogram from the *Sample solution* shows one red-violet zone turning to grayish-brown near the origin that corresponds in color and R_f value to that of *Standard solution A*, and one red-violet zone turning to grayish-brown at an R_f of 0.08 corresponding in color and R_f value to that in the chromatogram of *Standard solution B*; above these spots a grayish-brown zone may be present, corresponding in color and R_f value to that of *Standard solution A*; and other colored zones of varying intensities may be observed in the *Sample solution*.

• B. HPLC IDENTIFICATION TEST

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

Acceptance criteria: The chromatogram of the *Sample solution* presents a peak for docosyl ferulate that corresponds in retention time to the principal peak in the chromatogram of the *Standard solution*.

COMPOSITION

• CONTENT OF STEROLS

Derivatizing solution: Bis(trimethylsilyl)acetamide and trimethylchlorosilane (9:1)

Internal standard solution: 2 mg/mL of 5 α -cholestane in chloroform

System suitability stock solution: 2 mg/mL each of campesterol, stigmasterol, and USP β -Sitosterol RS

System suitability solution: Mix 2.0 mL of the *System suitability stock solution* and 2.0 mL of the *Internal standard solution*, and dilute with chloroform to 10 mL. Evaporate 500 μ L of this solution to dryness using a stream of nitrogen. Dissolve the residue in 80 μ L of *Derivatizing solution* and 20 μ L of pyridine. Allow to stand for NLT 10 min at room temperature.

Standard stock solution: 2.0 mg/mL of USP β -Sitosterol RS in chloroform

Standard solution: Mix 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution*, and dilute with chloroform to 10 mL. Evaporate 500 μ L of this solution to dryness using a stream of nitrogen. Dissolve the residue in 80 μ L of *Derivatizing solution* and 20 μ L of pyridine. Allow to stand for NLT 10 min at room temperature.

Sample solution: Transfer 100 mg of Extract into a 100-mL round-bottom flask. Add 2.0 mL of *Internal standard solution* and 20 mL of diluted hydrochloric acid. Attach a condenser, and reflux in a bath at 100° for 30 min. Cool the solution to room temperature, and adjust by the addition of about 5 mL of 10 N sodium hydroxide to a pH of 8. Extract twice using 50 mL of ether each time, wash the collected organic

phases with 50 mL of water, and evaporate the organic phase to dryness under vacuum. Dissolve the residue with 4 mL of chloroform, and transfer to a cartridge containing 500 mg of packing L8¹ that has been conditioned with a 2-column volume of *n*-hexane. Collect the eluate. Elute twice with a 1-column volume of a mixture of chloroform and isopropanol (2:1). Combine the eluates, and evaporate to dryness. Dissolve the residue in 10 mL of chloroform. Evaporate 500 µL of this solution to dryness under a stream of nitrogen. Dissolve the residue with 80 µL of *Derivatizing solution* and 20 µL of pyridine. Allow to stand for NLT 10 min at room temperature.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m capillary; G27 phase coating of 0.25-µm thickness

Temperature

Injector: 285°

Detector: 285°

Column: See [Table 1](#).

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
250	0	250	5
250	5	320	0

Carrier gas: Helium. [NOTE—The carrier gas flow rate should be adjusted to obtain a retention time of about 19 min for β-sitosterol.]

Makeup gas: Helium

Injection size: 2 µL

Injection type: Split injection system

Split ratio: 1:50

System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for 5α-cholestane, campesterol, stigmasterol, and β-sitosterol are about 0.66, 0.94, 0.96, and 1.00, respectively.]

Suitability requirements

Resolution: NLT 2 between campesterol and stigmasterol

Column efficiency: NLT 150,000 theoretical plates for the 5α-cholestane peak

Tailing factor: NMT 2.0 for each relevant peak

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the signals corresponding to the relevant analytes by comparison with the chromatograms obtained with the *System suitability solution*.

Separately calculate the individual percentages of campesterol, stigmasterol, and β-sitosterol, as β-sitosterol, respectively, in the portion of Pygeum Extract taken:

$$C_i = (R_U/R_S) \times C_S \times (V/W) \times 100$$

R_U = ratio of the appropriate sterol peak to the internal standard from the *Sample solution*

R_S = ratio of the β-sitosterol peak to the 5α-cholestane internal standard from the *Standard solution*

C_S = concentration of β-sitosterol in the *Standard stock solution* (mg/mL)

V = volume of the *Standard stock solution* taken to prepare the *Standard solution* (mL)

W = weight of Pygeum Extract taken to prepare the *Sample solution* (mg)