Saw Palmetto Extract

DEFINITION

Change to read:

Saw Palmetto Extract is obtained from comminuted Saw Palmetto by extraction with hydroalcoholic mixtures or solvent hexane, or by supercritical extraction with carbon dioxide. The ratio of starting crude plant material to Extract is from 8.0:1 to 14.3:1. •The Extract contains NLT 80.0% of fatty acids, NLT 0.2% of sterols, and NLT 0.1% of β-sitosterol, all on the anhydrous basis.• (RB 1-Jun-2013) The lipophilic Extract contains NLT 0.15% and NMT 0.35% of long-chain alcohols. The hydroalcoholic Extract contains NLT 0.01% and NMT 0.15% of long-chain alcohols. It contains no added substances.

IDENTIFICATION

Change to read:

- **A. Gas Chromatography**

Analysis: Proceed as directed in *Content of Fatty Acids*.

Acceptance criteria: The retention times of the 11 major peaks of the *Sample solution* correspond to those in the chromatogram of the *Standard solution*. •The ranges for ratios of the concentration of lauric acid to the concentration of the respective fatty acid in Extract prepared using hydroalcoholic mixtures or solvent hexane are in *Table 1*. The ranges for ratios of the concentration of lauric acid to the concentration of the respective fatty acid in Extract prepared by supercritical extraction with carbon dioxide are in *Table 2*.• (RB 1-Jun-2013)

Table 1

Fatty Acid	Minimum Ratio	Maximum Ratio
Capric	9.0	16
Caproic	8.5	24
Caprylic	8.5	17.5
Linoleic	5.0	16
Linolenic	31.5	55
Myristic	2.2	2.8
Oleic	0.60	1.15
Palmitic	2.8	3.9
Stearic	14	26

•**Table 2**

Fatty Acid	Minimum Ratio	Maximum Ratio
Capric	9.0	16
Caproic	9.0	40
Caprylic	8.5	17.5
Linoleic	4.0	8.0
Linolenic	35	60
Myristic	2.2	2.8
Oleic	0.60	1.15
Palmitic	2.8	3.9
Stearic	13	20

• (RB 1-Jun-2013)

COMPOSITION

Change to read:

- **Content of Fatty Acids**

Internal standard solution: 12 mg/mL of nonadecane in hexanes

Standard stock solution: Dissolve quantities of USP Methyl Laurate RS, USP Methyl Oleate RS, USP Methyl Myristate RS, USP Methyl Palmitate RS, USP Methyl Linoleate RS, USP Methyl Caproate RS, USP Methyl Caprylate RS, USP Methyl Caprate RS, USP Methyl Palmitoleate RS, USP Methyl Stearate RS, and USP Methyl Linolenate RS in hexanes to obtain concentrations of each methyl ester as given in *Table 3*.

Table 3

Methyl Ester	Concentration (mg/mL)
Methyl laurate	5
Methyl oleate	5
Methyl myristate	2
Methyl palmitate	2
Methyl linoleate	1
Methyl caproate	0.4
Methyl caprylate	0.4
Methyl caprate	0.4
Methyl palmitoleate	0.4
Methyl stearate	0.4
Methyl linolenate	0.4

Standard solution: Transfer 1.0 mL of the *Internal standard solution* to 5.0 mL of the *Standard stock solution*.

Sample solution: Transfer 100 mg of Extract to a pressure-proof, screw-capped vial, and add 3.0 mL of a solution of sulfuric acid in methanol (5 in 100). Heat at 100° in an oil bath for 2 h, shaking from time to time. Allow to cool, and add 1.0 mL of the *Internal standard solution*, 10.0 mL of water, 1 g of sodium chloride, and 5 mL of hexanes. Shake well, allow the layers to separate completely, and use the hexanes layer. [NOTE—Store in a refrigerator until ready to use.]

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 30-m fused silica capillary; 0.25-µm film of phase G16 coating
Temperatures
Injector: 250°
Detector: 300°
Column: See *Table 4*.

Table 4

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
120	0	120	3
120	50	220	12

Carrier gas: Helium
Flow rate: 1 mL/min
Injection volume: 1 µL
System suitability
Sample: *Standard solution*
[NOTE—See *Table 5* for the relative retention times.]

Table 5

Methyl Ester	Relative Retention Time
Methyl caproate	0.39
Methyl caprylate	0.56
Methyl caprate	0.76
Methyl laurate	0.94
Nonadecane (internal standard)	1.0
Methyl myristate	1.1
Methyl palmitate	1.3
Methyl palmitoleate	1.35
Methyl stearate	1.65
Methyl oleate	1.7
Methyl linoleate	1.8
Methyl linolenate	2.0

Suitability requirements
Resolution: NLT 1.5 between the methyl stearate and methyl oleate peaks
Tailing factor: NMT 2.0 for each of the methyl ester peaks
Relative standard deviation: NMT 5.0% for each of the methyl ester peaks
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of each fatty acid in the portion of Extract taken:

$$\text{Result} = (R_U/R_S) \times (C_S \times V) \times (1/W) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of the relevant methyl ester to the internal standard from the *Sample solution*
R_S = peak response ratio of the relevant methyl ester to the internal standard from the *Standard solution*
C_S = concentration of the respective methyl ester in the *Standard stock solution* (mg/mL)
V = volume of the *Standard stock solution* used to prepare the *Standard solution* (mL)
W = weight of Extract taken to prepare the *Sample solution* (mg)
M_{r1} = molecular weight of the relevant fatty acid
M_{r2} = molecular weight of the methyl ester of the relevant fatty acid

Acceptance criteria: •NLT 80.0% for the sum of the percentages of all the fatty acids, on the anhydrous basis.• (RB 1-Jun-2013)

Change to read:

• **CONTENT OF LONG-CHAIN ALCOHOLS AND STEROLS**
Derivatizing solution A: Bis(trimethylsilyl)acetamide, trimethylsilylimidazole, and trimethylchlorosilane (3:3:2)
Derivatizing solution B: *Derivatizing solution A*, bis(trimethylsilyl)trifluoroacetamide, and pyridine (1:1:1)
Internal standard solution: 10 mg/mL of eicosanol and 5 mg/mL of cholesterol in chloroform
System suitability stock solution A: 2 mg/mL each of tetracosanol, octacosanol, USP Hexacosanol RS, and triacontanol in chloroform
System suitability solution A: Mix 5.0 mL of *System suitability stock solution A* with 1.0 mL of the *Internal standard solution*. Evaporate 0.75 mL of this solution to dryness using a stream of nitrogen. Dissolve the residue in 1.0 mL of *Derivatizing solution B*, and allow to stand for NLT 15 min at room temperature.
System suitability stock solution B: 2 mg/mL each of campesterol, stigmasterol, and USP β-Sitosterol RS and 0.37 mg/mL of stigmastanol
System suitability solution B: Mix 5.0 mL of *System suitability stock solution B* with 1.0 mL of the *Internal standard solution*. Evaporate 0.75 mL of this solution to dryness using a stream of nitrogen. Dissolve the residue in 1.0 mL of *Derivatizing solution B*, and allow to stand for NLT 15 min at room temperature.
Standard stock solution: 0.75 mg/mL of USP Hexacosanol RS and 1.4 mg/mL of USP β-Sitosterol RS in chloroform
Standard solution: Mix 5.0 mL of the *Standard stock solution* with 1.0 mL of the *Internal standard solution*. Evaporate 0.75 mL of this solution to dryness using a stream of nitrogen. Dissolve the residue in 1.0 mL of *Derivatizing solution B*, and allow to stand for NLT 15 min at room temperature.
Sample solution: Transfer 3.35 g of Extract into a 50-mL round-bottomed flask. Add 1.0 mL of the *Internal standard solution*, and evaporate under vacuum at a temperature not exceeding 50°. Add 20 mL of a solution prepared by dissolving 130 g of potassium hydroxide in 200 mL of water in a 1000-mL volumetric flask, and dilute with methanol to volume. Attach a condenser, and reflux in a bath at 100° for 2 h. Quantitatively transfer this solution to a 25-mL volumetric flask, and dilute with water to volume. Transfer a 3-mL portion to a cartridge[1] containing diatomaceous earth capable of holding 3 mL of aqueous phase.
Absorb the solution into the column under vacuum for 20 min until the column is not cold. Elute the analytes from the column with 90 mL of methylene chloride, and evaporate the eluate to dryness. Dissolve the residue in 1.0 mL of *Derivatizing solution B*, and allow to stand for NLT 15 min at room temperature.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.2-mm × 25-m capillary; 0.33-µm thickness of phase G1 coating
Temperatures
Injector: 325°
Detector: 325°
Column: See *Table 6*.

Table 6

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
200	0	200	3
200	10	300	35

[1] A suitable cartridge is Extrelut NT3, or an equivalent cartridge.

Carrier gas: Helium
Flow rate: 0.5 mL/min
Make up gas flow: 25 mL/min
Injection volume: 1 µL
Injection type: Split ratio, 1:40

System suitability

Samples: *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for tetracosanol, hexacosanol, octacosanol, and triacontanol are 0.89, 1.00, 1.15, and 1.36, respectively, *System suitability solution A*. The relative retention times for cholesterol, campesterol, stigmasterol, β-sitosterol, and stigmastanol are 0.85, 0.92, 0.95, 1.00, and 1.01, respectively, *System suitability solution B*.]

Suitability requirements

Resolution: NLT 2 between the β-sistosterol and stigmastanol peaks, *System suitability solution B*

Column efficiency: NLT 200,000 theoretical plates for the eicosanol peak, *System suitability solution A*; and NLT 150,000 theoretical plates for the cholesterol peak, *System suitability solution B*

Tailing factor: NMT 2.0 for each relevant peak, *System suitability solution A*; and NMT 2.0 for each relevant peak, *System suitability solution B*

Analysis

Samples: *Standard solution* and *Sample solution*

Identify the signals corresponding to the relevant analytes by comparing the chromatograms obtained with *System suitability solution A* and *System suitability solution B*.

Separately calculate the percentages of tetracosanol, hexacosanol, octacosanol, and triacontanol, respectively, in the portion of Extract taken:

$$\text{Result} = (R_U/R_S) \times (C_S \times V) \times (1/W) \times 100$$

R_U = peak response ratio of the relevant long-chain alcohol to the internal standard from the *Sample solution*
R_S = peak response ratio of hexacosanol to the internal standard from the *Standard solution*
C_S = concentration of hexacosanol in the *Standard stock solution* (mg/mL)
V = volume of the *Standard stock solution* used to prepare the *Standard solution* (mL)
W = weight of the Extract taken to prepare the *Sample solution* (mg)

Calculate the total content of long-chain alcohols as a percentage by adding the individual percentages.

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

$$\text{Result} = (R_U/R_S) \times (C_S \times V) \times (1/W) \times 100$$

R_U = peak response ratio of the relevant sterol to the internal standard from the *Sample solution*
R_S = peak response ratio of β-sitosterol to the 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* used to prepare the *Standard solution* (mL)
W = weight of the Extract taken to prepare the *Sample solution* (mg)

Calculate the total content of sterols as a percentage by adding the individual percentages.

Acceptance criteria: •NLT 0.2% for the sum of the percentages of all the sterols and NLT 0.1% of β-sitosterol, both on the anhydrous basis• (RB 1-Jun-2013)

CONTAMINANTS

- **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 40 µg/g
- **BOTANICAL EXTRACTS,** *Pesticide Residues* ⟨**565**⟩**:** Meets the requirements

SPECIFIC TESTS

- **ALCOHOL DETERMINATION,** *Method II* ⟨**611**⟩ (if present)**:** NMT 1%
- **FATS AND FIXED OILS,** *Iodine Value* ⟨**401**⟩**:** 40–50
- **FATS AND FIXED OILS,** *Saponification Value* ⟨**401**⟩**:** 210–250

Delete the following:

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** 1.8%–3.5% (RB 1-Jun-2013)
- **WATER DETERMINATION,** *Method I* ⟨**921**⟩**:** NMT 3% is found in the hydroalcoholic Extract.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Meets the requirements in *Botanical Extracts* ⟨565⟩*, Packaging and Storage*
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of fatty acids and sterols and the ratio of the starting crude plant material to Extract. It meets the requirements in *Botanical Extracts* ⟨565⟩*, Labeling*.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Hexacosanol RS
  USP Methyl Caprate RS
  USP Methyl Caproate RS
  USP Methyl Caprylate RS
  USP Methyl Laurate RS
  USP Methyl Linoleate RS
  USP Methyl Linolenate RS
  USP Methyl Myristate RS
  USP Methyl Oleate RS
  USP Methyl Palmitate RS
  USP Methyl Palmitoleate RS
  USP Methyl Stearate RS
  USP β-Sitosterol RS