Total ash (*2.4.16*): maximum 10.0 per cent.

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

ASSAY

Tannins (*2.8.14*). Use 0.500 g of the powdered herbal drug (180) (*2.9.12*).

01/2014:2579

SAW PALMETTO EXTRACT

Sabalis serrulatae extractum

DEFINITION

Extract produced from *Saw palmetto fruit (1848)*.

Content:

- *total fatty acids*: minimum 80.0 per cent (anhydrous extract);
- *lauric acid* ($C_{12}H_{24}O_2$; M_r 200.3): minimum 23.0 per cent (anhydrous extract);
- *total sterols, expressed as β-sitosterol* ($C_{29}H_{50}O$; M_r 414.7): minimum 0.20 per cent (anhydrous extract);
- *β-sitosterol* ($C_{29}H_{50}O$; M_r 414.7): minimum 0.10 per cent (anhydrous extract).

PRODUCTION

The extract is produced from the herbal drug by a suitable procedure using ethanol (minimum 90 per cent *V/V*), or supercritical carbon dioxide or a mixture of mainly *n*-hexane and methylpentanes (bp: 65-70 °C).

CHARACTERS

Appearance: the ethanol extract is a dark greenish-brown, oily liquid; the supercritical carbon dioxide extract is a yellowish-brown or orange-brown, oily liquid; the hexane extract is a yellowish-green to orange-yellow, oily liquid.

Odour: strong but not rancid.

IDENTIFICATION

First identification: *B*.

Second identification: *A*.

A. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 0.25 g of the extract to be examined in 20 mL of *ethanol (96 per cent) R*.

Reference solution. Dissolve 4 mg of *β-amyrin R* and 10 mg of *β-sitosterol R* in 10 mL of *ethanol (96 per cent) R*.

Plate: *TLC silica gel plate R* (2-10 µm).

Mobile phase: *anhydrous acetic acid R*, *ethyl acetate R*, *toluene R* (1:30:70 *V/V/V*).

Application: 2 µL as bands of 8 mm.

Development: over a path of 6 cm.

Drying: in air.

Detection: treat with *anisaldehyde solution R*, heat at 100-105 °C for 5-10 min and examine in daylight.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones may be present, especially in the lower third, in the chromatogram obtained with the test solution.

Top of the plate	
	A blue zone
	A blue zone
_____	_____
β-Amyrin: a blue zone	A strong bluish-violet zone
β-Sitosterol: a blue zone	
_____	_____
Reference solution	**Test solution**

B. Examine the chromatograms obtained in the assay of total fatty acids.

Results: the peaks due to methyl caproate, methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitoleate, methyl palmitate, methyl linoleate, methyl linolenate, methyl oleate and methyl stearate in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (b); the principal peaks are due to methyl laurate and methyl oleate.

TESTS

Water (*2.5.12, Method A*): maximum 3.0 per cent, determined on 0.5 g.

Relative density (*2.2.5*): 0.850 to 0.950.

Refractive index (*2.2.6*): 1.40 to 1.50.

Acid value (*2.5.1*): 150.0 to 220.0.

Iodine value (*2.5.4, Method A*): 30.0 to 60.0.

Peroxide value (*2.5.5*): maximum 5.0.

Saponification value (*2.5.6*): 220.0 to 250.0.

Solvents. Residual solvents are controlled as described in chapter *5.4*, unless otherwise justified and authorised.

ASSAY

Total fatty acids. Gas chromatography (*2.2.28*).

Internal standard solution. Dissolve 0.47 g of *methyl margarate R* in 20 mL of *dimethylformamide R* and dilute to 100.0 mL with the same solvent.

Test solution. Disperse 0.25 g of the extract to be examined in 10 mL of *dimethylformamide R*. Add 4.0 mL of the internal standard solution and dilute to 25.0 mL with *dimethylformamide R*. Mix 0.4 mL of this solution and 0.6 mL of a 18.84 g/L solution of *trimethylsulfonium hydroxide R* in *methanol R*.

Reference solution (a). Dissolve 0.699 g of *lauric acid CRS* and 0.870 g of *oleic acid CRS* in *dimethylformamide R* and dilute to 10.0 mL with the same solvent. To 1.0 mL of the solution add 4.0 mL of the internal standard solution and dilute to 25.0 mL with *dimethylformamide R*. Mix 0.4 mL of this solution and 0.6 mL of a 18.84 g/L solution of *trimethylsulfonium hydroxide R* in *methanol R*.

Reference solution (b). Disperse 0.25 g of *saw palmetto extract HRS* in 10 mL of *dimethylformamide R*. Add 4.0 mL of the internal standard solution and dilute to 25.0 mL with *dimethylformamide R*. Mix 0.4 mL of this solution and 0.6 mL of a 18.84 g/L solution of *trimethylsulfonium hydroxide R* in *methanol R*.

Column:

- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.20 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.33 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 0.5 mL/min.

Split ratio: 1:40.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 2	150
	2 - 7	150 → 190
	7 - 12	190
	12 - 22	190 → 220
	22 - 32	220
Injection port		300
Detector		300

Detection: flame ionisation.

Injection: 1 µL.

Identification of peaks: use the chromatogram supplied with *saw palmetto extract HRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to methyl caproate, methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitoleate, methyl palmitate, methyl linoleate, methyl linolenate, methyl oleate, methyl stearate and methyl margarate.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to methyl linolenate and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to methyl linoleate.

Calculate the percentage content of total fatty acids, where caproic, caprylic, capric, lauric, myristic, palmitoleic, palmitic and stearic acids are expressed as lauric acid ($C_{12}H_{24}O_2$; M_r 200.3) and linoleic, linolenic and oleic acids are expressed as oleic acid ($C_{18}H_{34}O_2$; M_r 282.5), using the following expression:

$$\frac{A_1 \times A_4 \times m_2 \times p_1 \times 0.1}{A_2 \times A_3 \times m_1} + \frac{A_5 \times A_4 \times m_3 \times p_2 \times 0.1}{A_6 \times A_3 \times m_1}$$

A_1 = sum of the areas of the peaks due to methyl caproate, methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitoleate, methyl palmitate and methyl stearate in the chromatogram obtained with the test solution;

A_2 = area of the peak due to methyl laurate in the chromatogram obtained with reference solution (a);

A_3 = area of the peak due to methyl margarate in the chromatogram obtained with the test solution;

A_4 = area of the peak due to methyl margarate in the chromatogram obtained with reference solution (a);

A_5 = sum of the areas of the peaks due to methyl linoleate, methyl linolenate and methyl oleate in the chromatogram obtained with the test solution;

A_6 = area of the peak due to methyl oleate in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *lauric acid CRS* used to prepare reference solution (a), in grams;

m_3 = mass of *oleic acid CRS* used to prepare reference solution (a), in grams;

p_1 = percentage content of lauric acid in *lauric acid CRS*;

p_2 = percentage content of oleic acid in *oleic acid CRS*.

Calculate the percentage content of lauric acid using the following expression:

$$\frac{A_1 \times A_4 \times m_2 \times p \times 0.1}{A_2 \times A_3 \times m_1}$$

A_1 = area of the peak due to methyl laurate in the chromatogram obtained with the test solution;

A_2 = area of the peak due to methyl laurate in the chromatogram obtained with reference solution (a);

A_3 = area of the peak due to methyl margarate in the chromatogram obtained with the test solution;

A_4 = area of the peak due to methyl margarate in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *lauric acid CRS* used to prepare reference solution (a), in grams;

p = percentage content of lauric acid in *lauric acid CRS*.

Total sterols. Gas chromatography (*2.2.28*).

Derivatisation solution (a): *chlorotrimethylsilane R*, *N,O-bis(trimethylsilyl)acetamide R*, *N-trimethylsilylimidazole R* (2:3:3 *V/V/V*).

Derivatisation solution (b): derivatisation solution (a), *N,O-bis(trimethylsilyl)trifluoroacetamide R*, *pyridine R* (1:1:1 *V/V/V*).

Internal standard solution. Dissolve 0.25 g of *cholesterol R* in 25.0 mL of *methylene chloride R*.

Test solution. Introduce 1.0 mL of the internal standard solution into a 50 mL round-bottomed flask and evaporate to dryness. Place 3.35 g of the extract to be examined, accurately weighed, into the round-bottomed flask and add 20 mL of a solution prepared as follows: dissolve 130 g of *potassium hydroxide R* in 200 mL of *water R* and dilute to 1000 mL with *methanol R*. Heat under reflux for 2 h, transfer quantitatively to a flask and dilute to 25.0 mL with *water R*. Apply 3.0 mL of this solution to a cartridge containing *diatomaceous earth R* capable of holding 3 mL of aqueous phase. Absorb the solution into the column by applying vacuum. Maintain the vacuum for at least 20 min, until the column returns to room temperature, indicating that the methanol is completely evaporated. Rinse the column with 90 mL of *methylene chloride R* and evaporate the eluate to dryness. Dissolve the residue in 1.0 mL of derivatisation solution (b).

Reference solution (a). To 9.0 mg of *β-sitosterol CRS* add 1.0 mL of the internal standard solution and dilute to 5.0 mL with *methylene chloride R*. Evaporate 0.6 mL of this solution to dryness under a stream of *nitrogen R*. Dissolve the residue in 1.0 mL of derivatisation solution (b).

Reference solution (b). Introduce 1.0 mL of the internal standard solution into a 50 mL round-bottomed flask and evaporate to dryness. Place 3.35 g of *saw palmetto extract HRS*, accurately weighed, into the round-bottomed flask and add 20 mL of a solution prepared as follows: dissolve 130 g of *potassium hydroxide R* in 200 mL of *water R* and dilute to 1000 mL with *methanol R*. Heat under reflux for 2 h, transfer quantitatively to a flask and dilute to 25.0 mL with *water R*. Apply 3.0 mL of this solution to a cartridge containing *diatomaceous earth R* capable of holding 3 mL of aqueous phase. Absorb the solution into the column by applying vacuum. Maintain the vacuum for at least 20 min, until the column returns to room temperature, indicating that

the methanol is completely evaporated. Rinse the column with 90 mL of *methylene chloride R* and evaporate the eluate to dryness. Dissolve the residue in 1.0 mL of derivatisation solution (b).

Column:

- *material*: fused silica;
- *size*: *l* = 25 m, Ø = 0.20 mm;
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.33 µm).

Carrier gas: *helium for chromatography R.*

Flow rate: 0.5 mL/min.

Split ratio: 1:40.

	Time (min)	Temperature (°C)
Column	0 - 3	200
	3 - 13	200 → 300
	13 - 35	300
Injection port		325
Detector		325

Detection: flame ionisation.

Injection: 1 µL.

Identification of peaks: use the chromatogram supplied with *saw palmetto extract HRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to the trimethylsilyl derivatives of cholesterol, campesterol, stigmasterol, β-sitosterol and stigmastanol.

System suitability: reference solution (b):

- *resolution*: minimum 1.6 between the peaks due to the trimethylsilyl derivatives of β-sitosterol and stigmastanol.

Calculate the percentage content of total sterols (campesterol, stigmasterol, β-sitosterol and stigmastanol), expressed as β-sitosterol, using the following expression:

$$\frac{A_1 \times A_4 \times m_2 \times p}{A_2 \times A_3 \times m_1}$$

A_1 = sum of the areas of the peaks due to the trimethylsilyl derivatives of campesterol, stigmasterol, β-sitosterol and stigmastanol in the chromatogram obtained with the test solution;

A_2 = area of the peak due to the trimethylsilyl derivative of β-sitosterol in the chromatogram obtained with reference solution (a);

A_3 = area of the peak due to the trimethylsilyl derivative of cholesterol in the chromatogram obtained with the test solution;

A_4 = area of the peak due to the trimethylsilyl derivative of cholesterol in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *β-sitosterol CRS* used to prepare reference solution (a), in grams;

p = percentage content of β-sitosterol in *β-sitosterol CRS*.

Calculate the percentage content of β-sitosterol using the following expression:

$$\frac{A_1 \times A_4 \times m_2 \times p}{A_2 \times A_3 \times m_1}$$

A_1 = area of the peak due to the trimethylsilyl derivative of β-sitosterol in the chromatogram obtained with the test solution;

A_2 = area of the peak due to the trimethylsilyl derivative of β-sitosterol in the chromatogram obtained with reference solution (a);

A_3 = area of the peak due to the trimethylsilyl derivative of cholesterol in the chromatogram obtained with the test solution;

A_4 = area of the peak due to the trimethylsilyl derivative of cholesterol in the chromatogram obtained with reference solution (a);

m_1 = mass of the extract to be examined used to prepare the test solution, in grams;

m_2 = mass of *β-sitosterol CRS* used to prepare reference solution (a), in grams;

p = percentage content of β-sitosterol in *β-sitosterol CRS*.

07/2012:1848

SAW PALMETTO FRUIT

Sabalis serrulatae fructus

DEFINITION

Dried ripe fruit of *Serenoa repens* (W. Bartram) Small (syn. *Sabal serrulata* (Michaux) T. Nuttal ex Schultes & Schultes).

Content: minimum 11.0 per cent of total fatty acids (dried drug).

CHARACTERS

Odour: strong but not rancid.

IDENTIFICATION

First identification: A, B, D.

Second identification: A, B, C.

A. The fruit is an ovoid or subspherical drupe, with a dark brown or blackish, roughly wrinkled surface and more or less coppery sheen, up to 2.5 cm long and 1.5 cm in diameter. The apex sometimes bears the remains of the style and tubular calyx, with 3 teeth, and the base bears a small depression with the scar of the stalk. The epicarp and underlying mesocarp form a thin fragile layer, which partially peels off, revealing the thin, hard, pale brown endocarp, which is fibrous and easily separable. The seed is irregularly spherical or ovoid, up to 12 mm long and 8 mm in diameter, with a hard, smooth or finely pitted surface which is reddish-brown with a paler, raised and membranous area over the raphe and micropyle; cut transversely, the seed has a thin testa, narrow perisperm and a large area of dense, horny, greyish-white endosperm, with the embryo positioned to one side.

B. Microscopic examination (*2.8.23*). Reduce to a powder (710) (*2.9.12*). The powder is reddish or blackish-brown and oily. Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of epicarp composed of several layers of thin-walled, reddish-brown, pigmented, polyhedral cells (10-40 µm) which are strongly cuticularised; those of the outer layers are much smaller than those of the inner layers. Parenchyma cells of the mesocarp may be large and filled with oil droplets, or smaller and containing nodules of silica. Groups of xylem tissue of the mesocarp show small lignified, annular or spirally thickened vessels. Stone cells of the mesocarp (20-200 µm) may be found scattered, usually singly but sometimes in small groups, the walls are moderately thickened, distinctly striated and finely pitted. Fragments of endocarp contain groups of elongated