AMERICAN SPIKENARD FOR HOMOEOPATHIC PREPARATIONS

ARALIA RACEMOSA FOR HOMOEOPATHIC PREPARATIONS

Aralia racemosa ad praeparationes homoeopathicas

DEFINITION

Dried, underground part of *Aralia racemosa* L.

Content: minimum 0.10 per cent of chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3) (dried drug).

IDENTIFICATION

A. Fleshy, fibrous rhizome, whitish to pale brown outside, irregularly cylindrical, tuberous in places, ramified measuring up to 40 cm long and usually 10-50 mm in diameter. More or less ramified roots, 5-25 mm in diameter and measuring up to 25 cm long, inserted at the level of the annular nodes. More or less concave scars on the upper part of the rhizome, due to the fall of the leaves.

B. Reduce the underground part to a powder (355). The powder is yellowish-brown. Examine under a microscope, using *chloral hydrate solution R.* The powder presents scarce fragments of suber composed of superposed polyhedral cells; fragments of parenchyma consisting of ovoid cells, some of them containing calcium oxalate clusters; secretory canals, most often fragmented; fragments of pitted or reticulate vessels; calcium oxalate clusters. Examine under a microscope using *glycerol* (50 per cent *V/V*) *R*. The powder presents starch granules; spherical and isolated, about 10 µm in diameter.

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

Test solution. Add 30 mL of *ethanol* (65 per cent *V/V*) *R* to 3 g of powdered drug (355). Heat under a reflux condenser at 60 °C for 15 min. Allow to cool. Filter.

Reference solution. Dissolve 5 mg of *chlorogenic acid R* and 5 mg of *caffeic acid R* in 20 mL of *ethanol* (*96 per cent*) *R.*

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V*).

Application: 20 µL [or 10 µL] as bands.

Development: over a path of 10 cm [or 6 cm].

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

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

Top of the plate	
	A blue zone
Caffeic acid: a blue zone	
-----	-----
Chlorogenic acid: a greenish-blue zone	A greenish-blue zone (chlorogenic acid)
-----	-----
	A greenish-blue zone
Reference solution	**Test solution**

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

Test solution. Add 30 mL of *ethanol* (65 per cent *V/V*) *R* to 3 g of powdered drug (355). Heat under a reflux condenser at 60 °C for 15 min. Allow to cool. Filter.

Reference solution. Dissolve 10 mg of *oleanolic acid R* and 10 mg of *cholesterol R* in 30 mL of *ethanol* (*96 per cent*) *R.*

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *acetone R*, *methylene chloride R* (10:90 *V/V*).

Application: 30 µL [or 20 µL] as bands.

Development: over a path of 10 cm [or 7 cm].

Drying: in air.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. 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 in the chromatogram obtained with the test solution.

Top of the plate	
	A purplish zone
	An intense, spread out violet zone
Cholesterol: a purple zone	
	A purplish-blue zone
	A more or less intense purplish-blue zone
-----	-----
Oleanolic acid: a purplish-pink zone	
	A purplish zone
-----	-----
	A blue zone
	A purplish zone
Reference solution	**Test solution**

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

TESTS

Loss on drying (*2.2.32*): maximum 11.0 per cent, determined on 1.0 g of powdered drug (355) by drying in an oven at 105 °C for 2 h.

Total ash (*2.4.16*): maximum 10.0 per cent, determined on 1.0 g of powdered drug (355).

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Place 2.500 g of powdered drug (355) and 40 mL of *ethanol* (60 per cent *V/V*) *R* into a 250 mL flask. Heat under a reflux condenser for 30 min. Allow to separate then filter into a 100.0 mL volumetric flask. Add 40 mL of *ethanol* (60 per cent *V/V*) *R* to the residue and heat again under a reflux condenser for 30 min. Filter, rinse the flask and the filter with *ethanol* (60 per cent *V/V*) *R* and transfer into the 100.0 mL volumetric flask. After cooling, dilute to 100.0 mL with *ethanol* (60 per cent *V/V*) *R.*

Reference solution. In a 100.0 mL volumetric flask, dissolve 20.0 mg of *chlorogenic acid CRS* and 20.0 mg of *rosmarinic acid R* in *ethanol* (60 per cent *V/V*) *R* and dilute to 100.0 mL with the same solvent. Take 7.0 mL of this solution and dilute to 20.0 mL with *ethanol* (60 per cent *V/V*) *R.*

Column:
- *size*: *l* = 0.25 m, *Ø* = 4 mm,
- *stationary phase*: *end-capped, octylsilyl silica gel for chromatography R* (5 µm); porosity 10 nm, specific surface 350 m²/g, carbon rate 12.5 %,
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *glacial acetic acid* (10 per cent *V/V*) *R,*
- *mobile phase B: methanol R.*

Time (min)	**Mobile phase A (per cent *V/V*)**	**Mobile phase B (per cent *V/V*)**
0 - 10	100 → 0	0 → 100
10 - 20	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 326 nm.

Injection: 10 µL. Retention time of chlorogenic acid: about 6 min, of rosmarinic acid: about 8 min.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

System suitability:
- *resolution*: minimum 5 between the peaks due to chlorogenic acid and rosmarinic acid in the chromatogram obtained with the reference solution.

Calculate the percentage content of chlorogenic acid, from the expression:

$$\frac{A_1 \times m_2 \times 0.35 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution,
A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution,
m_1 = mass of the drug sample, in grams,
m_2 = mass of the sample of chlorogenic acid, in grams,
p = percentage content of chlorogenic acid in *chlorogenic acid CRS.*

STOCK

DEFINITION

American spikenard mother tincture is prepared with ethanol (65 per cent *V/V*), using the dried underground part of *Aralia racemosa* L.

Content: minimum 0.006 per cent *m/m* of chlorogenic acid ($C_{16}H_{18}O_9$; M_r 354.3).

PRODUCTION

*Method 1.1.10 (2371).*Drug fragmented into 2-6 cm long segments. Maceration time: 3-5 weeks.

CHARACTERS

Appearance: yellow liquid.

Specific fragrance remembering wax.

IDENTIFICATION

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

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *chlorogenic acid R* and 5 mg of *caffeic acid R* in 20 mL of *ethanol* (*96 per cent*) *R.*

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (10:10:80 *V/V/V*).

Application: 20 µL [or 10 µL] as bands.

Development: over a path of 10 cm [or 7 cm].

Drying: in air.

Detection: examine in ultraviolet light at 365 nm.

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

Top of the plate	
Caffeic acid: a blue zone --- Chlorogenic acid: a greenish-blue zone ---	A blue zone --- A greenish-blue zone (chlorogenic acid) --- A greenish-blue zone
Reference solution	**Test solution**

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

Test solution. Mother tincture.

Reference solution. Dissolve 10 mg of *oleanolic acid R* and 10 mg of *cholesterol R* in 30 mL of *ethanol* (*96 per cent*) *R.*

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: *acetone R, methylene chloride R* (10:90 *V/V*).

Application: 30 µL [or 10 µL] as bands.

Development: over a path of 10 cm [or 7 cm].

Drying: in air.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min. 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 in the chromatogram obtained with the test solution.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

Top of the plate	
Cholesterol: a purple zone ----- Oleanolic acid: a purplish-pink zone ----- 	A purplish zone An intense, spread out violet zone A purplish-blue zone ----- A purplish zone ----- A blue zone A purplish zone
Reference solution	**Test solution**

TESTS

Ethanol (*2.9.10*): 60 per cent *V/V* to 70 per cent *V/V.*

Dry residue (*2.8.16*): minimum 1.5 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 20.0 mL volumetric flask, place 10.00 g of mother tincture and dilute to 20.0 mL with *ethanol* (60 per cent *V/V*) *R*. Filter.

Reference solution. In a 100.0 mL volumetric flask, dissolve 20.0 mg of *chlorogenic acid CRS* and 20.0 mg of *rosmarinic acid R* in *ethanol* (60 per cent *V/V*) *R* and dilute to 100.0 mL with the same solvent. Take 5.0 mL of this solution and dilute to 20.0 mL with *ethanol* (60 per cent *V/V*) *R.*

Column:
- *size*: *l* = 0.25 m, Ø = 4. mm,
- *stationary phase*: *end-capped, octylsilyl silica gel for chromatography R* (250 x 4 mm, 5 µm), porosity 10 nm, specific surface 350 m²/g, carbon rate 13 %.
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: *glacial acetic acid* (10 per cent *V/V*) *R.*
- *mobile phase B*: *methanol R.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100 → 0	0 → 100
10 - 20	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 326 nm.

Injection: 10 µL.

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.

System suitability:

- *resolution:* minimum 5 between the peaks due to chlorogenic acid and rosmarinic acid in the chromatogram obtained with the reference solution.

Calculate the percentage content *m/m* of chlorogenic acid, from the expression:

$$\frac{A_1 \times m_2 \times 0.05 \times p}{A_2 \times m_1}$$

A_1 = area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution,
A_2 = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution,
m_1 = mass of the mother tincture sample, in grams,
m_2 = mass of the sample of chlorogenic acid, in grams,
p = percentage content of chlorogenic acid in *chlorogenic acid CRS.*

The General Chapters and General Monographs of the European Pharmacopoeia and Preamble of the French Pharmacopoeia apply.