

## MUGWORT FOR HOMOEOPATHIC PREPARATIONS ARTEMISIA VULGARIS FOR HOMOEOPATHIC PREPARATIONS

### *Artemisia vulgaris* ad praeparationes homoeopathicas

#### DEFINITION

Fresh, underground part of *Artemisia vulgaris* L.

#### IDENTIFICATION

Underground organ resembling a tape root. Brown, cylindrical roots up to 3 cm in diameter with a fibrous and whitish fracture. Grooved cylindrical stolons, dark brown, up to 2 cm in diameter. Adventitious roots, dark brown, spindly with a fibrous fracture and whose diameter does not exceed 2 mm, located at the top of the main root, at the level of each leaf scar.

#### TESTS

**Foreign matter** (2.8.2): maximum 5 per cent.

**Loss on drying** (2.2.32): minimum 50.0 per cent, determined on 5.0 g of finely-cut drug by drying in an oven at 100-105 °C for 2 h.

### STOCK

#### DEFINITION

Mugwort mother tincture is prepared with ethanol (65 per cent *V/V*), using the fresh, underground organ of *Artemisia vulgaris* L.

*Content*: minimum 0.010 per cent *m/m* of total hydroxycinnamic derivatives, expressed as chlorogenic acid (C<sub>16</sub>H<sub>18</sub>O<sub>9</sub>; *M<sub>r</sub>* 354.3).

#### PRODUCTION

*Method 4c* (2371). Drug fragmented into segments 3-4 cm long. Maceration time: 3-5 weeks.

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**2004-2010.**

## CHARACTERS

Yellow liquid.

## IDENTIFICATION

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

*Test solution.* Mother tincture.

*Reference solution.* Dissolve 10 mg of *chlorogenic acid R* and 20 mg of *rutin R* in 40 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*, *methyl ethyl ketone R*, *ethyl acetate R* (10:10:30:50 V/V/V/V).

*Application:* 20 µl [or 10l as bands].

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

*Drying:* in air.

*Detection:* first spray with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R* then with a 50 g/l solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry for about 30 min. 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	
----- Chlorogenic acid: a bluish-green zone Rutin: an orange zone -----	A greenish-blue zone A greenish-yellow zone A greenish-yellow zone ----- A bluish-green zone (chlorogenic acid) -----
<b>Reference solution</b>	<b>Test solution</b>

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

*Test solution.* Shake 10 ml of mother tincture with 3 quantities, each of 20 ml of *hexane R*. Filter the organic phases and evaporate them to dryness, under reduced pressure at about 30 °C. Dissolve the residue in 3 ml of *ethanol (96 per cent) R*.

*Reference solution.* Dissolve 2 mg of *anethole R* and 2 µl of *cineole R* in 10 ml of *methanol R*.

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

*Mobile phase :* *ethyl acetate R, toluene R* (7:93 *V/V*).

*Application :* 50 µl [or 30 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	
Anethole : a purplish zone -----	A violet zone Two brown to purple zones -----
Cineole : a purplish zone -----	A violet to pink zone ----- Two violet to brown zones
<b>Reference solution</b>	<b>Test solution</b>

## TESTS

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

**Methanol and 2-propanol** (2.9.11) : maximum 0.05 per cent *V/V*; maximum 0.05 per cent *V/V*.

**Dry residue** (2.8.16) : minimum 1.0 per cent *m/m*.

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## ASSAY

Liquid chromatography (2.2.29).

*Test solution.* Place 10.00 g of mother tincture into a 20.0 ml volumetric flask and dilute to 20.0 ml with *ethanol* (60 per cent *V/V*) *R*.

*Reference solution.* In a 100.0 ml volumetric flask, dissolve 10.0 mg of *chlorogenic acid CRS* and 10.0 mg of *rosmarinic acid R* in *ethanol* (60 per cent *V/V*) *R* and dilute to 100.0 ml with the same solvent.

*Column :*

- *size* :  $l = 0.25$  m,  $\varnothing = 4$  mm,
- *stationary phase* : end-capped octylsilyl silica gel for chromatography (5  $\mu$ m); porosity 10 nm; specific surface 350 m<sup>2</sup>/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 <i>V/V</i> )	Mobile phase B (per cent <i>V/V</i> )
0-15 15-20	100 → 0 0	0 → 100 100

*Flow rate* : 1.0 ml/min.

*Detection* : spectrophotometer at 326 nm.

*Injection* : 10  $\mu$ l.

*Retention time* : chlorogenic acid = about 7 min ; rosmarinic acid = about 10 min.

*Relative retention* : of the main derivative in comparison with chlorogenic acid : 1.8.

*System suitability :*

- *resolution* : minimum 7 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 hydroxycinnamic derivatives, expressed as chlorogenic acid from the expression :

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$$\frac{(A_1 + A_2) \times m_2 \times p}{A_3 \times m_1 \times 5}$$

$A_1$  = area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution,

$A_2$  = area of the major peak due to relative retention 1.8 in comparison with the peak of chlorogenic acid in the chromatogram obtained with the test solution,

$A_3$  = 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 chlorogenic acid sample, in grams,

$p$  = percentage content of chlorogenic acid in *chlorogenic acid CRS*.

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