

## ABSINTHIUM FOR HOMOEOPATHIC PREPARATIONS

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**Artemisia absinthium ad praeparationes homoeopathicas**  
Other Latin name used in homoeopathy: **Artemisia absinthium**

### DEFINITION

Fresh, flowering aerial part of *Artemisia absinthium* L.

### CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.  
Strong, penetrating odour.

### IDENTIFICATION

- A. Absinth possesses ramose, angular silver-grey branches with inner pith. It has alternate, petiolate leaves. The lower leaves are twice to thrice pinnately divided and have lanceolate segments. The upper leaves are simpler, becoming entire and linear. The leaves are whitish on both sides. The inflorescence consists of a large, leafy panicle with erect branches and nutant capitula, 3 - 4 mm in diameter. The whitish involucre is made up of imbricated scales. The yellow flowers are tubular and fertile. The peripheral flowers are arranged in a single row and have a 3-toothed corolla; the central flowers have a 5-toothed corolla. The receptacle is covered with long, white hairs.
- B. Take a sample of epidermis from the underside of the leaf. Examine under a microscope using *chloral hydrate solution R*. The upper epidermis contains stomata and has both secretory and covering trichomes. The covering trichomes are lozenge-shaped with generally a bi-cellular base. The secretory trichomes are either sessile, with a biseriate pluricellular head (typical of Asteraceae) or uniseriate and pluricellular; the distal cell is slightly larger than the base cells.

### TESTS

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

**Loss on drying** (2.2.32): minimum 55.0 per cent, determined on 5.0 g of finely cut drug, by drying in an oven at 105 °C for 2 h.

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

***Artemisia arborescens* L.** The drug has non-ligneous stems at the base and no erect capitula when in flower.

## STOCK

### DEFINITION

Absinth mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement).

The mother tincture is prepared with ethanol (65 per cent V/V), using the fresh flowering aërial part of *Artemisia absinthium* L.

*Content:* minimum 0.05 per cent *m/m* of total dihydroxycinnamic derivatives, expressed as chlorogenic acid ( $C_{16}H_{18}O_9$ ;  $M_r$  354.3).

### CHARACTERS

*Appearance:* brownish-green liquid.

### IDENTIFICATION

A. Distil 10 g of mother tincture. To the distillate, add 2 mL of *zinc sulfate solution R* and 0.5 mL of *sodium nitroprusside solution R*. Shake, then add 4 mL of *diluted carbon dioxide-free sodium hydroxide solution R*. After a few minutes, add 2-3 mL of *glacial acetic acid R*. An orange-red colour appears which then turns purple-brown (absinthine).

B. Thin-layer chromatography (2.2.27).

*Test solution.* Mother tincture.

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

*Plate:* TLC silica gel plate *R*.

*Mobile phase:* *water R*, *anhydrous formic acid R*, *methyl ethyl ketone R*, *ethyl formiate R*, *ethyl acetate R*, *toluene R* (5:10:15:20:25:25 V/V/V/V/V).

*Application:* 30 µL, as bands.

*Development:* over a path of 10 cm.

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*Drying:* in air.

*Detection A:* examine in ultra violet light at 365 nm.

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

<b>Top of the plate</b>	
Caffeic acid: a blue zone	Two red zones A blue zone
Chlorogenic acid: a blue zone	A blue zone A blue zone (chlorogenic acid)
<b>Reference solution</b>	<b>Test solution</b>

*Detection B:* spray with a 10 g/L solution of *diphenylboric acid aminoethyl ester R* in *methanol R*. Then, spray a 50 g/L solution of *macrogol 400 R* in *methanol R*. Allow the plate to dry for about 30 min. Examine in ultra violet light at 365nm.

*Results B:* 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.

<b>Top of the plate</b>	
Caffeic acid: a green zone	A green zone A greenish-blue zone
Chlorogenic acid: a greenish-blue zone	A greenish-blue zone A greenish-blue zone (chlorogenic acid)
<b>Reference solution</b>	<b>Test solution</b>

## TESTS

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

**Dry residue:** minimum 1.0 per cent *m/m* (see French Pharmacopoeia Authority Supplement).

**Thujone:** maximum 0.1 per cent *m/m*.

Calculate the thujone content by gas chromatography (2.2.28).

**Internal standard solution.** Dissolve 0.02 g of (1*S*-*cis*)-2-carene *R* in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent.

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

*Test solution.* To 5.00 g of mother tincture, add 2.0 mL of internal standard solution and dilute to 10.0 mL with *ethanol (96 per cent) R*.

*Reference solution.* Dissolve 0.10 g of *thujone R* in *ethanol (96 per cent) R* and dilute to 100.0 mL with the same solvent. To 5.0 mL of solution, add 2.0 mL of internal standard solution and dilute to 10.0 mL with *ethanol (96 per cent) R*.

*Column:*

- *material:* fused silica,
- *size:*  $l=30$  m,  $\varnothing = 0.53$  mm,
- *stationary phase:* *dimethylsiloxane R* (film thickness: 1.5  $\mu\text{m}$ ).

*Carrier gas:* *helium for chromatography R*.

*Flow rate:* 24 mL/min.

*Temperature:*

- *column:* 60°C
- *temperature program:* as follows:

	Time (min)	Temperature (°C)
Column	0 — 10	60
	10 — 20	60 → 70
	20 — 25	70
	25 — 32	70 → 240
	32 — 40	240
Injection port		250
Detector		250

*Detection:* flame ionisation.

*Injection:* 2  $\mu\text{L}$ .

Calculate the sum of the  $\alpha$ -thujone and  $\beta$ -thujone contents of the mother tincture, from the expression:

$$\frac{A_{EI} \times A_E \times m_T \times 5}{A'_{EI} \times A_T \times m_E}$$

$A_{EI}$  = area of the peak corresponding to the internal standard in the reference solution,

$A'_{EI}$  = area of the peak corresponding to the internal standard in the test solution,

$A_T$  = sum of the areas of the peaks corresponding to  $\alpha$ -thujone and  $\beta$ -thujone in the reference solution,

$A_E$  = sum of the areas of the peaks corresponding to  $\alpha$ -thujone and  $\beta$ -thujone in the test solution,

$m_T$  = mass of the thujone sample, in grams,

$m_E$  = mass of the mother tincture sample, in grams.

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

## ASSAY

Ultraviolet and visible absorption spectrophotometry (2.2.25).

*Test solution (a).* In a 25.0 mL volumetric flask, place 5.00 g of mother tincture and dilute to 25.0 mL with *ethanol (50 per cent V/V) R*.

*Test solution (b).* In a 10.0 mL volumetric flask, add successively and shake after each addition: 1.0 mL of test solution (a), 2 mL of *0.5M hydrochloric acid R*, 2 mL of a solution containing 100 g/L of *sodium nitrite R* and 100 g/L of *sodium molybdate R*, 2 mL of *dilute sodium hydroxide R*. Dilute to 10.0 mL with *water R*.

*Compensation liquid.* Place the following in a 10.0 mL volumetric flask: 1.0 mL of test solution (a), 2 mL of *0.5M hydrochloric acid*, 2 mL of *dilute sodium hydroxide R*. Dilute to 10.0 mL with *water R*.

Measure the absorbance of test solution (b) immediately at 525 nm, by comparison with the compensation liquid.

Calculate the percentage content *m/m* of total dihydroxycinnamic derivatives calculated as chlorogenic acid, from the expression:

$$\frac{A \times 1.33}{m}$$

i.e. taking the specific absorbance of chlorogenic acid at 525 nm to be 188

*A* = absorbance of test solution (b) at 525 nm,

*m* = mass of the mother tincture sample, in grams