BIRTHWORT
FOR HOMOEOPATHIC PREPARATIONS

ARISTOLOCHIA CLEMATITIS
FOR HOMOEOPATHIC PREPARATIONS

Aristolochia clematitis ad praeparationes homoeopathicas

DEFINITION

Fresh, flowering, aerial parts of *Aristolochia clematitis* L.

CHARACTERS

Macroscopic and microscopic characters described under identification tests A and B.

IDENTIFICATION

A. Erect, angulous stem about 80 cm high. Alternate, cordiform, petioled leaves, green on the upper side and paler on the underside, entire and palmately veined. Groups of 6 or 8 flowers at the axil of the leaves, greenish-yellow, with a tubular perianth, swollen at the base, enlarged and strip-like at the top. Androecium with 6 stamens. Sub-sessile anthers, with backs joined in pairs to the style and displayed in a crown. Inferior ovary with 6 multi ovulated loculi. Short style ended with a 6-curved-lobe stigma.

B. Examine a fragment of underside epidermis from the leaf under a microscope, using *chloral hydrate solution R*. Epidermis covered with a thin cuticle finely punctuated. Epidermis from the veins, forming a network of rectangular to parallelipipedic cells lined along the veins. Within the meshes of the net, epidermis from the lamina composed of slightly-lobed cells, deep in the epidermis stomata of anomocytic type (*2.8.3*) with 4 to 6 subsidiary cells, small, ovoid, sessile, unicellular covering trichomes.

TESTS

Foreign matters (*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 105 °C for 2 h.

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

STOCK

DEFINITION

Birthwort 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 (45 per cent *V/V*), using fresh, flowering, aerial parts of *Aristolochia clematitis* L.

Adjusted content: minimum 0.010 per cent and maximum 0.030 per cent *m/m* of aristolochic acids [aristolochic acid I ($C_{17}H_{11}NO_7$; M_r 341.3) and aristolochic acid II ($C_{16}H_9NO_6$; M_r 311.3)].

CHARACTERS

Appearance: reddish-brown liquid.

IDENTIFICATION

Thin layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *aristolochic acid R1* and 2 mg of *chlorogenic acid R* in *ethanol (96 per cent) R* and complete to 10 mL with the same solvent.

Plate: *TLC silica gel plate R.*

Mobile phase: *glacial acetic acid R, water R, butanol R* (10:10:40 *V/V/V*).

Application: 20 µL, as bands.

Development: over a path of 10 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.

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

Top of the plate	
Aristolochic acid: a dark zone ------ Chlorogenic acid: a greenish-blue zone ------	A dark zone (aristolochic acid) An intense greenish-blue zone ------ A more or less intense orange zone ------
Reference solution	**Test solution**

TESTS

Ethanol (*2.9.10*): 40 per cent *V/V* to 50 per cent *V/V*.

Dry residue (*2.8.16*): minimum 1.8 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. In a 20.0 mL volumetric flask, place 10.000 g of mother tincture and dilute to 20.0 mL with *methanol R*.

Reference solution. In a 200.0 mL volumetric flask, dissolve 15.0 mg of *aristolochic acid R1* in *methanol R* and dilute to 200.0 mL with the same solvent.

Column:
- *size*: *l* = 0.25 m, Ø = 4 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
- *temperature*: 30 °C.

Mobile phase:
- *mobile phase A*: a mixture of 1 volume of *glacial acetic acid R* and 40 volumes of *water R,*
- *mobile phase B*: *methanol R.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 25	50 → 40	50 → 60
25 – 30	40 → 0	60 → 100
30 – 35	0	100
35 – 40	0 → 50	100 → 50

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 250 nm.

Injection: 10 µL.

Retention time: aristolochic acid II about 17 min and aristolochic acid I about 22 min.

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

Elution order: aristolochic acid II, aristolochic acid I.

System suitability: reference solution.
- *resolution:* minimum 3 between the peaks of aristolochic acid II and aristolochic acid I.

Calculate the percentage content *m/m* of aristolochic acids with the total content of aristolochic acid I and aristolochic acid II, from the expression:

$$\frac{A_1 \times m_2 \times 10}{A_2 \times m_1}$$

A_1 = areas of the peaks corresponding to aristolochic acid I and aristolochic acid II in the chromatogram obtained with the test solution,
A_2 = areas of the peaks corresponding to aristolochic acid I and aristolochic acid II in the chromatogram obtained with the reference solution,
m_1 = mass of the mother tincture sample, in grams,
m_2 = mass of aristolochic acid in the reference solution, in grams.

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