GUACO FOR HOMOEOPATHIC PREPARATIONS

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Mikania guaco ad praeparationes homoeopathicas

DEFINITION

Dried leaf of *Mikania guaco* H. et B. (*Mikania amara* Willd.).

Content: minimum 0.1 per cent of coumarin ($C_9H_6O_2$; M_r 146.1) (dried drug).

CHARACTERS

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

IDENTIFICATION

A. Guaco leaf is simple, oval, entire, acuminate at the apex and rounded at the base. It is leathery and glabrous to slightly pubescent on the adaxial surface. The veins are prominent on the abaxial surface. 2-4 secondary veins emerge from the base of the lamina. The leaf is 16-24 cm long and 11 cm wide. The 5 cm long petiole is circular in section and glabrous or pubescent.

B. Reduce the leaf to a powder (355). The powder is brown. When examined under a microscope using *chloral hydrate solution R*, the following are observed: fragments of abaxial lamina epidermis consisting of lobed or polyhedral cells, glandular trichomes and anisocytic stomata (*2.8.3*) with lacunate parenchyma; fragments of adaxial laminar epidermis covered with a smooth cuticle, consisting of lobed or polyhedral cells and glandular trichomes with palisade parenchyma; fragments of leaf veins with vessels arranged in spirals or rings. Two types of glandular trichomes are found: some with multi cellular stalk and inverted unicellular head, others are sessile with multi cellular, biseriate head.

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

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

Test solution. To 3 g of powdered herbal drug (355), add 30 ml of *ethanol R (65 per cent V/V)*. Cover. Heat to 60 °C on a water-bath for 15 min. Allow to cool. Filter.

Reference solution. Dissolve 1 mg of *scopoletin R* and 1 mg of *coumarin R* in 10 ml of *ethanol (96 per cent) R*.

Plate: *TLC silica gel plate R*.

Mobile phase: upper phase of a mixture of *dilute acetic acid R, ether R, toluene R* (10:50:50 *V/V/V*).

Application: 5 µl, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R*. 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	
Coumarin: a green-blue zone Scopoletin: a pale blue zone	A green-blue zone (coumarin) A green-blue zone
Reference solution	**Test solution**

TESTS

Foreign matter (*2.8.2*): it complies with the test for foreign matter.

Loss on drying (*2.2.32*): maximum 12.0 per cent, determined on 1.000 g of powdered herbal drug (355), by drying in an oven at 100-105 °C for 2 h.

Total ash (*2.4.16*): maximum 12.0 per cent.

Aristolochic acid.

Thin-layer chromatography (*2.2.27*).

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

Test solution. To 3 g of powdered herbal drug (355), add 10 ml of *ethanol R (65 per cent V/V)*. Cover. Heat to 60 °C on a water-bath for 15 min. Allow to cool. Filter.

Reference solution. Dissolve 5 mg of *aristolochic acid R1* in *ethanol (96 per cent) R* and dilute to 20 ml with the same solvent.

Plate: *TLC silica gel plate F_{254} R*.

Mobile phase: *acetone R, methanol R, chloroform R* (10:10:60 *V/V/V*).

Application: 20 µl, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 254 nm.

Results A: see below the sequence of fluorescent quenching zones present in the chromatograms obtained with the reference solution and test solutions. No zone corresponding to the aristolochic acid zone in the reference solution is present in the chromatogram obtained with the test solution.

Top of the plate	
	A dark zone
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	A dark zone
------	------
Aristolochic acid: a dark zone	Absence of zone
Reference solution	**Test solution**

Detection B: examine in ultraviolet light at 365 nm.

Results B: see below the sequence of fluorescent zones present in the chromatograms obtained with the reference solution and the test solution. No zone corresponding to the aristolochic acid zone in the reference solution is present in the chromatogram obtained with 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.

2003.

Top of the plate	
	A red zone Two blue zones A reddish zone
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	An intense green-blue zone
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Aristolochic acid: a brown zone	Absence of zone
Reference solution	**Test solution**

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. To 1.500 g of powdered herbal drug (355), add 45 ml of *ethanol R (65 per cent V/V)* and heat under a reflux condenser for 1 h. Allow to cool. Filter through a fibre-glass filter. Dissolve the residue and the fragmented filter in 45 ml of *ethanol R (65 per cent V/V)*. Treat as above. Combine the filtrates and dilute to 100.0 ml with *ethanol R (65 per cent V/V)*.

Reference solution. Dissolve 15.0 mg of *coumarin R* in *ethanol R (65 per cent V/V)* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 20.0 ml with *ethanol R (65 per cent V/V)*.

Column:

— *size*: l = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm),

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: *in 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	80	20
25-30	80 → 0	20 → 100
30-40	0	100

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 280 nm.

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

Injection: 10 µl.

System suitability: reference solution.

— *Retention time* of coumarin: about 20 min.

Calculate the percentage content of coumarin, from the expression:

$$\frac{A_1 \times m_2 \times 25}{A_2 \times m_1}$$

A_1 = peak area for coumarin in the test solution chromatogram,

A_2 = peak area for coumarin in the reference solution chromatogram,

m_1 = mass of the herbal drug sample in grams,

m_2 = mass of coumarin in the reference solution in grams.

STOCK

DEFINITION

Guaco 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 dried leaf of *Mikania guaco* H. et B.

Content: minimum 0.01 per cent *m*/*m* of coumarin ($C_9H_6O_2$; M_r 146.1).

CHARACTERS

Ochre-green liquid.

Characteristic odour.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 1 mg of *scopoletin R* and 1 mg of *coumarin R* in 10 ml of *ethanol (96 per cent) R*.

Plate: *TLC silica gel plate R*.

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

2003.

Mobile phase: upper phase of a mixture of *dilute acetic acid R, ether R, toluene R* (10:50:50 *V/V/V*).

Application: 5 μl, as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with a 100 g/l solution of *potassium hydroxide R* in *methanol R*. Examine in ultraviolet light at 365 nm.

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

Top of the plate	
Coumarin: a green-blue zone Scopoletin: a pale blue zone	A green-blue zone (coumarin) A green-blue zone
Reference solution	**Test solution**

TESTS

Aristolochic acid: absence.

Liquid chromatography (*2.2.29*).

Test solution. Mother tincture.

Reference solution. Dissolve 10.0 mg of *aristolochic acid R1* in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of this solution to 20.0 ml with *methanol R*.

Column:

— *size*: l = 0.25 m, Ø = 4.0 mm,

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm),

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: in a mixture of 1 volume of *glacial acetic acid R* and 40 volumes of *water R*,

— *mobile phase B*: *methanol R*.

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

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

Flow rate: 1.0 ml/min.

Detection: spectophotometer (with diode array detector) at 250 nm.

Injection: 20 µl.

Order of elution: aristolochic acid II, aristolochic acid I.

System suitability: reference solution.

— *Resolution*: minimum 5 between the peaks due to aristolochic acid II and aristolochic acid I.

— *Detection threshold*: 1 ppm for the sum of aristolochic acid II and aristolochic acid I peak areas.

— *Signal-to-noise ratio*: above 3 for a concentration of 1 ppm *aristolochic acid R1*.

Plot the absorption spectrum (220-450 nm) of the aristolochic acid I and aristolochic acid II peaks obtained with the reference solution.

Plot the absorption spectrum (220-450 nm) of any peak(s) obtained with the test solution if occurring at the retention time(s) of the aristolochic acid I and/or II peaks from the reference solution and with a peak area of at least 0.1 times that of the corresponding peak(s) obtained with the reference solution.

The spectra of the peaks obtained with the test solution and the spectra of the aristolochic acid I and/or II peaks obtained with the reference solution should not overlap.

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 0.5 per cent *m/m*.

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

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Dilute 3.000 g of mother tincture to 20.0 ml with *ethanol R (65 per cent V/V)*.

Reference solution. Dissolve 15.0 mg of *coumarin R* in *ethanol R (65 per cent V/V)* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 20.0 ml with *ethanol R (65 per cent V/V)*.

Column:

— *size*: l = 0.25 m, Ø = 4.0 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	80	20
25-30	80 → 0	20 → 100
30-40	0	100

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 280 nm.

Injection: 10 μl.

System suitability: reference solution.

— *Retention time* of coumarin: about 20 min.

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

$$\frac{A_1 \times m_2 \times 5}{A_2 \times m_1}$$

A_1 = peak area for coumarin in the test solution chromatogram,

A_2 = peak area for coumarin in the reference solution chromatogram,

m_1 = mass of the mother tincture sample in grams,

m_2 = mass of coumarin in the reference solution in grams.

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