ARNICA (WHOLE PLANT)
FOR HOMOEOPATHIC PREPARATIONS

ARNICA MONTANA
FOR HOMOEOPATHIC PREPARATIONS

Arnica montana ad praeparationes homoeopathicas

DEFINITION

Whole, fresh, blooming plant *Arnica montana* L.

IDENTIFICATION

A. Plant 20-60 cm high, evergreen thanks to a thick rootstock, cylindrical, radicate, oblique and slightly creeping. Radial, light green leaves, entire, sessile, oval, lanceolate, displayed in a rosette flattened on the ground; lamina slightly dentate; main rib usually ramified into five secondary, parallel and prominent ribs. Under and upper sides scattered with short hairs and secretory trichomes. Annual, floral stem, hairy, erect, ending in a single capitulum; one or two pairs of caulinary leaves, opposite, entire, sessile, shorter than those of the rosette. Sometimes a pair of secondary twigs, each bearing a capitulum at the axil of the upper leaves. Orange-yellow, spread out capitulum whose diameter may reach 6-8 cm long. Capitulum surrounded by an involucre consisting of 18-24 lanceolate bracts, elongated with acute apices, displayed on 1 or 2 rows. About 20 female, peripheral flowers, ligulate, inserted on a single row, 20-30 mm long; 3-toothed ligula. More numerous hermaphrodite, tubular mid flowers, corolla ending in 5 teeth; calyx reduced to a crown of hairs inserted on a single row. Five stamens, surrounding a style with 2 stigmatic branches curving outwards. Possible presence of ribbed achenes, 6.5-9 mm long, topped by a pappus of bristles displayed in a single row and of length quite similar to the corolla's.

B. Take a sample of epidermis from the leaf. Examine under a microscope, using *chloral hydrate solution R*: underside epidermis of the lamina consisting of cells with sinuous outline and numerous stomata of anomocytic type (*2.8.3*); straight, multicellular covering trichomes with thickened cell-walls and tapering end and very scarce secretory trichomes, multicellular, biseriate of Asteraceae-type.

TESTS

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

Loss on drying (*2.2.32*): minimum 75.0 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

Arnica montana (whole plant) mother tincture is prepared with ethanol (45 per cent *V/V*) using the whole, fresh, blooming plant *Arnica montana* L.

Content : minimum 0.01 per cent *m/m* of sesquiterpene lactones expressed as dihydrohelenalin tiglate ($C_{20}H_{26}O_5$; M_r 346.4).

PRODUCTION

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

CHARACTER

Appearance: yellowish-brown liquid.

IDENTIFICATION

Thin layer chromatography (*2.2.27*).

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *caffeic acid R,* 5 mg of *chlorogenic acid R* and 10 mg of *rutin R* in 40 mL of *ethanol* (*96 per cent*) *R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R*, *water R, methyl ethyl ketone R, ethyl acetate R* (10:10:30:50 *V/V/V/V).*

Application: 40 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 spray 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.

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

Top of the plate	
Caffeic acid: a greenish-blue zone ----- Chlorogenic acid: a bluish zone ----- Rutin: an orange zone	A greenish-blue zone ----- A bluish zone A yellowish-brown to orange-yellow 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 1.5 per cent *m/m*.

ASSAY

Liquid chromatography (*2.2.29*).

Internal standard solution. Immediaely before use, dissolve 0.010 g of s*antonin R,* accurately weighed and 0.02 g of *4-hydroxybenzoate butyl R* in 10.0 mL of *methanol R.*

Test solution. In a round-bottomed flask, place 25.000 g of mother tincture, add 2.0 mL of internal standard solution and 15 g of *neutral aluminium oxide R.* Shake for 2 min and filter. Rinse the flask and the filter 3 times, each time with 5 mL of a mixture of equal volumes of *methanol R* and *water R.* Evaporate the filtrate to dryness under reduced pressure, at a temperature below 50 °C. Dissolve the residue in 2.0 mL of a mixture of 80 volumes of *methanol R* and 20 volumes of *water R* then filter.

Reference solution. In a 10.0 mL volumetric flask, dissolve 0.020 g of *4-hydroxybenzoate methyl R* and 0.020 g of *4-hydroxybenzoate ethyl R* in *methanol R* and dilute to 10.0 mL with the same solvent.

Column:
– *size*: *l* = 0.125 m, *Ø* = 4 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm),
– *temperature*: 30 °C.

Mobile phase:
– *mobile phase A*: *water R,*
– *mobile phase B*: *methanol R.*

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 – 3	62	38
3 – 20	62 → 55	38 → 45
20 – 30	55	45
30 – 55	55 → 45	45 → 55

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Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 225 nm.

Injection: 20 µL.

Relative retention time in comparison with santonin (retention time = about 9.5 min):
4-hydroxybenzoate butyl = about 4.6.

System suitability: reference solution:
– *resolution*: minimum 5.0 between the peaks due to *4-hydroxybenzoate methyl* and 4- hydroxybenzoate ethyl.

Calculate the percentage content *m/m* of sesquiterpene lactones expressed as dihydrohelenalin tiglate from the expression:

$$\frac{A_1 \times C \times V \times 1{,}187}{A_2 \times m \times 10}$$

A_1 = sum of the areas of the peaks between the peaks due to santonin and to *4-hydroxybenzoate butyl* in the chromatogram obtained with the test solution,
A_2 = area of the peak due to santonin in the chromatogram obtained with the test solution,
m = mass of the mother tincture sample, in grams,
C = concentration in santonine of the internal standard solution used for the test solution in milligrams per millilitre,
V = volume of the internal standard solution used for the test solution, in millilitres,
1.187 = correlation factor between dihydrohelenalin tiglate and santonin.

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