SPRING PHEASANT'S EYE FOR HOMOEOPATHIC PREPARATIONS

ADONIS VERNALIS FOR HOMOEOPATHIC PREPARATIONS

Adonis vernalis ad praeparationes homoeopathicas

DEFINITION

Fresh, flowering aerial part of cultivated *Adonis vernalis* L.

IDENTIFICATION

A. Grooved stem, 15-20 cm high; slightly ramified, some stems are sterile, others bear flowers. Radical leaves reduced to scales. Caulinary leaves, sessile, shortly sheathing and glabrous, with palmatisect lamina divided into thin strips, carved at the base into 7 segments, themselves divided into a various number of secundary narrow segments shaping thin strips. Solitary flowers at the apex of twigs with 5 greenish, oval sepals and 12-18 oval petals, pale yellow, slightly striated, spread out; numerous stamens, receptacle bearing numerous free carpels.

B. Examine a fragment of leaf under a microscope, using *chloral hydrate solution R*: lower epidermis of the lamina composed of cells with very sinuous outlines and numerous anomocytic stomata (*2.8.3*); lower epidermis of the midrib composed of rectangular cells with regularly thickened cell-walls, elongated along the midrib; margin of the lamina bearing unicellular, elongated secretory trichomes, oboval, 30 µm long.

TESTS

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

Loss on drying (*2.2.32*): minimum 60.0 per cent, determined on 5.0 g of finely-cut drug (355) by drying in an oven at 105 °C for 2 h.

STOCK

DEFINITION

Spring pheasant's eye mother tincture is prepared with ethanol (45 per cent *V/V*), using the fresh, flowering aerial part of cultivated *Adonis vernalis* L.

Content: minimum 0.01 per cent *m/m* and maximum 0.03 per cent *m/m* of total cardiotonic heterosides, expressed as cymarin ($C_{30}H_{44}O_9$; M_r 548.7).

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

PRODUCTION

Method 1.1.10 (2371). Drug fragmented into segments 5-10 cm long. Maceration time: 3-5 weeks.

CHARACTERS

Appearance: greenish-brown liquid.

IDENTIFICATION

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

Test solution. Mother tincture.

Reference solution. Dissolve 5 mg of *luteolol-7 glucoside R* and 10 mg of *rutin R* in 20 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: *glacial acetic acid R, water R, butanol R* (10:10:40 *V/V/V*).

Application: 20 µL [or 10 µL] as bands.

Development: over a path of 10 cm [or 6 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	
Luteolol-7 glucoside: an orange zone	
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	A yellow zone
Rutin: an orange zone	An orange-yellow zone
	A yellow zone
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	A series of blue zones
Reference solution	**Test solution**

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

Test solution. Heat to boiling 20 mL of mother tincture with 5 mL of *lead acetate solution R,* for 2 min. After cooling centrifuge the mixture. Shake the supernatant with 30 mL of *methylene chloride R.* Collect the organic solution and dry it upon *anhydrous sodium sulfate R.* Filter. Evaporate to dryness 15 mL of this solution on a water-bath. Dissolve the residue in 0.5 mL of *ethanol* (*96 per cent*) *R*.

Reference solution. Dissolve 10 mg of convallatoxin *R* and 10 mg of *cymarin 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: *water R, methanol R, ethyl acetate R* (8:11:81 *V/V/V*).

Application: 40 µL [or 20 µL] as bands.

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

Drying: in air.

Detection: spray with *dinitrobenzoic acid solution R*, then with a 100 g/L solution of *potassium hydroxide R* in *methanol R*. 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, fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Cymarin: a purple zone Convallatoxin: a purple zone	A purple zone A purple zone A purple 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 1.0 per cent *m/m*.

ASSAY

Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Stock solution. In a centrifugation tube, place 20.000 g of mother tincture, add 12 mL of *lead basic acetate solution R.* Shake then centrifuge. Collect the supernatant in a 50.0 mL volumetric flask. Add 5 mL of *water R* through the opening of centrifugation. Shake. Centrifuge. Transfer the

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supernatant into a 50.0 mL volumetric flask. Repeat the operation once more. Dilute to 50.0 mL with *water R.* Take 10.0 mL of the solution and add 10.0 mL of a 100 g/L solution of *sodium sulfate R*. Filter.

Test solution. Add 2.0 mL of *dinitrobenzoic acid solution R* to 10.0 mL of stock solution and 1.0 mL of *1 M sodium hydroxide R.* Shake.

Reference stock solution. In a 100.0 mL volumetric flask, dissolve 10.0 mg of *cymarin R* in 20 mL of *ethanol* (*96 per cent*) *R* and dilute to 100.0 mL with *water R.* In a 20.0 mL volumetric flask, place 5.0 mL of this solution and dilute to 20.0 mL with *water R*.

Reference solution. Add 2.0 mL of *dinitrobenzoic acid solution R* to 10.0 mL of reference stock solution and 1.0 mL of *1 M sodium hydroxide R.* Shake.

Compensation liquid. Add 2.0 mL of *dinitrobenzoic acid solution R* to 10.0 mL of *water R* and 1.0 mL of *1 M sodium hydroxide R.* Shake.

Within the first 12 min, measure the absorbance of the test solution and of the reference solution several times, at 540 nm, in comparison with the compensation liquid until the maximum is reached.

Calculate the percentage content *m/m* of total cardiotonic heterosides, expressed as cymarin from the expression:

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

A_1 = maximum absorbance of the test solution,
A_2 = maximum absorbance of the reference solution,
m_1 = mass of the mother tincture sample, in grams,
m_2 = mass of cymarin sample, in grams.

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