EYEBRIGHT
FOR HOMOEOPATHIC PREPARATIONS

EUPHRASIA OFFICINALIS
FOR HOMOEOPATHIC PREPARATIONS

Euphrasia stricta and Euphrasia rostkoviana ad praeparationes homoeopathicas

DEFINITION

Whole, fresh, flowering plant *Euphrasia stricta* D. Wolff ex F.J. Lehm. and/or *Euphrasia rostkoviana* Hayne and/or their hybrids and/or their mixtures.

CHARACTERS

Macroscopic characters described under identification.

IDENTIFICATION

Main root and rootlets bearing lateral suckers, each one composed of a small nipple whose top is more or less truncated and surrounded by a membranous crown forming a kind of suction disc. Erect or ascending stem, 5-25 cm high, ramose and bearing small, sessile leaves, opposite at the base and alternate near the apex. Greyish-green stiff, oval leaves markedly dentate; both sides covered with glandular trichomes. Flowers displayed in leafy racemes quite loose; appearing at the axil of foliaceous, dentate, pubescent bracts. Pubescent, tubular calyx, ending with 4 triangular teeth. White corolla much longer than the calyx, striated with mauve or violet, tubular and bilabiate; upper lip bilobed, erect like a helmet; lower lip trilobed with a yellowish throat. Two of the four stamens are longer and hairy; their anthers show 2 mucronate lobes at the base. Ovary containing 2 loculi; the style is thread-like.

TESTS

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

Loss on drying (*2.2.32*): minimum 65.0 per cent, determined on 5.0 g of finely-cut drug by drying in an oven at 105 °C for 2 h.

STOCK

DEFINITION

Eyebright mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homeopathic Preparations (1038)* and French Pharmacopoeia

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

Supplement). The mother tincture is prepared with ethanol (55 per cent *V/V*), using the whole, fresh, flowering plant *Euphrasia stricta* D. Wolff ex F.J. Lehm. and/or *Euphrasia rostkoviana* Hayne and/or their hybrids and/or their mixtures.

Content: minimum 0.03 per cent *m/m* of aucubin ($C_{15}H_{22}O_9$; M_r 346.3).

CHARACTERS

Appearance: brown liquid.

IDENTIFICATION

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

Test solution. Add 10 mL of *water R* to 10 mL of mother tincture. Shake twice with 10 mL of *ethyl acetate R*. Dry the organic phase on *anhydrous sodium sulfate R* and filter. Evaporate to dryness and dissolve the residue in 1 mL of *methanol R*.

Reference solution. Dissolve 10 mg of *rutin R* and 10 mg of *hyperoside R* in 20 mL of *ethanol (96 per cent) R.*

Plate: *TLC silica gel plate R*.

Mobile phase: *anhydrous formic acid R, water R, ethyl acetate R* (8:8:84 *V/V/V*).

Application: 20 µL as bands.

Development: over a path of 15 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	
	Two hardly separated orange zones
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	A pale orange zone
Hyperoside: an orange zone	A greenish-yellow zone
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	Two greenish-yellow zones
Rutin: an orange zone	An orange zone (rutin)
Reference solution	**Test solution**

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

Test solution. Mother tincture.

Reference solution. Dissolve 10 mg of *aucubin R* and 10 mg of *harpagoside R* in 20 mL of *ethanol (96 per cent) R.*

Plate: *TLC silica gel plate R.*

Mobile phase: *glacial acetic acid R, anhydrous formic acid R, water R, ethyl acetate R* (11:11:27:100 *V/V/V/V*).

Application: 20 µL as bands.

Development: over a path of 10 cm.

Drying: in air.

Detection: spray with *anisaldehyde solution R*, heat at 100-105 °C for 5 min. 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 zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
	One to two greenish zones
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Harpagoside: a brown zone	
	Two purplish zones
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Aucubin: a purplish-grey zone	A purplish-grey zone (aucubin) One to two greenish zones
Reference solution	**Test solution**

TESTS

Ethanol (*2.9.10*): 50 per cent *V/V* to 60 per cent *V/V.*

Dry residue (*2.8.16*): minimum 1.2 per cent *m/m.*

ASSAY

Liquid chromatography (*2.2.29*).

Test solution. Place 5.000 g of mother tincture into a flask. Evaporate to dryness, under reduced pressure at a temperature below 40 °C. Dilute the residue with 20 mL of mixture of 1 volume of

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water R and 2 volumes of *methanol R*. Lay down the whole quantity on a column of neutral *aluminium oxide R*, about 10 cm high and 1.2 cm in diameter. Elute with the same solvent until 150 mL are obtained with the aid of vacuum. Evaporate the eluate to dryness. Dilute the residue with a few millilitres of mobile phase, transfer into a 10.0 mL volumetric flask and dilute to 10.0 mL with the same solvent.

Reference solution (a). In a 100.0 mL volumetric flask, dissolve 0.020 g of *aucubin R* in *5*0 mL of mobile phase and dilute to 100.0 mL with the same solvent.

Reference solution (b). In a 20.0 mL volumetric flask, dissolve 5.0 mg of *catalpol R* and dilute to 20.0 mL with the mobile phase. Take 1.0 mL of this solution and add 2.0 mL of reference solution (a).

Column:
– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *acetonitrile R1*, *water R* (3:97 *V/V*).

Flow rate: 0.5 mL/min.

Detection: spectrophotometer at 204 nm.

Injection: 10 µL. Retention time of aucubin: about 19 min.

System suitability: reference solution (b).
– *Resolution*: minimum 4.5 between the peaks due to catalpol and aucubin.

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

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

A_1 = area of the peak due to aucubin in the chromatogram obtained with the test solution,
A_2 = area of the peak due to aucubin in the chromatogram obtained with the reference solution (a),
m_1 = mass of the mother tincture sample, in grams,
m_2 = mass of *aucubin R* in the reference solution (a), in grams.

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