

**BLUEBELL
FOR HOMOEOPATHIC PREPARATIONS**

**AGRAPHIS NUTANS
FOR HOMOEOPATHIC PREPARATIONS**

Hyacinthoides non-scripta ad praeparationes homoeopathicas

Other Latin name used in homoeopathy: **Endymion nutans**

DEFINITION

Whole, fresh, flowering plant, *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. [*Endymion non-scriptus* (L.) Garcke, *E. nutans* (L.) Dum., *Agraphis nutans* Link.].

CHARACTERS

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

IDENTIFICATION

- A. Bluebell is a perennial plant with a floral scape, 20-40 cm high with an ovoid, tunicated bulb. The base flowers are elongated, 6-15 mm large, erect then spreading, often shorter than the stem. The floral scape ends by a unilateral raceme of fragrant, blue flowers, sometimes white. The flowers are pendent at the tip of a pedicel shorter than the flowers and bearing at its base 2 small, lanceolate bracts the same colour as the flowers. The perianth, compressed at the base, measures up to 18 mm. Among the 6 stamens, 3 have fused filaments, linked to the petals over half their length, the other 3 are shorter and almost free.
- B. Take a sample of abaxial epidermis of the leaf of bluebell. Examine under a microscope, using *chloral hydrate solution R*. The abaxial epidermis is stomatiferous and covered with a finely striated cuticle; the striations are parallel and following the longitudinal orientation of the epidermic cells. The epidermic cells are very narrow and elongated; they are about 10 µm large and more than 300 µm long. The stomata are surrounded by 4 subsidiary cells. Among them, 2 are located on either side of the stoma, parallel to the ostiole; the remaining 2 are located on each end of the stoma. These subsidiary cells are similar to the other epidermic cells. Free, calcium oxalate raphides are frequent.

TESTS

Foreign matter (2.8.2): maximum 5 per cent.

Loss on drying (2.2.32): minimum 70.0 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

Bluebell mother tincture complies with the requirements of the general technique for the preparation of the mother tincture (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Supplement). The mother tincture is prepared with ethanol (45 per cent V/V), using the whole, fresh, flowering plant *Hyacinthoides non-scripta* (L.) Chouard ex Rothm.

Content: minimum 0.01 per cent *m/m* of total flavonoids, expressed as apigenin (C₁₅H₁₀O₅; M_r 270.2).

CHARACTERS

Appearance: yellow liquid.

IDENTIFICATION

Thin-layer chromatography (2.2.27).

Test solution. Mother tincture.

Reference solution. Dissolve 10 mg of *rutin R* and 10 mg of *apigenin-7-glucoside R* in 20 mL of *ethanol* (60 per cent V/V) *R*.

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 and the test solutions. 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	
Apigenin-7-glucoside: a green zone -----	A yellow zone -----
Rutin: an orange zone -----	A greenish-yellow zone A greenish-yellow zone A yellow zone A 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

Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. Evaporate 1.000 g of mother tincture to dryness, under reduced pressure. Dilute the residue in 25.0 mL of a mixture composed of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* (solution 1). To 10.0 mL of this solution, add 10.0 mL of a 25 g/L *boric acid R* and 20 g/L *oxalic acid R* solution in *anhydrous formic acid R* then dilute to 25.0 mL with *glacial acetic acid R*.

Compensation liquid 1. In a 25.0 mL volumetric flask, place 10.0 mL of solution 1, add 10.0 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R*.

Reference solution. Dissolve 15.0 mg of *apigenin R* in a mixture composed of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and dilute to 100.0 mL with the same mixture (solution 2). In a 25.0 mL volumetric flask, place 1.0 mL of this solution, add 9 mL of a mixture composed of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and 10.0 mL of a 25 g/L *boric acid R* and 20 g/L *oxalic acid R* solution in *anhydrous formic acid R* then dilute to 25.0 mL with *glacial acetic acid R*.

Compensation liquid 2. In a 25.0 mL volumetric flask, place 1.0 mL of solution 2, add 9 mL of a mixture composed of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R* and 10.0 mL of *anhydrous formic acid R* then dilute to 25.0 mL with *glacial acetic acid R*.

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Thirty min later, measure the absorbance (2.2.25) of the test solution at 400 nm, in comparison with compensation liquid 1 and the absorbance of the reference solution in comparison with compensation liquid 2.

Calculate the percentage content m/m of total flavonoids, expressed as apigenin, from the expression:

$$\frac{A_1 \times m_2 \times 2.5}{A_2 \times m_1}$$

A_1 = absorbance of the test solution at 400 nm,

A_2 = absorbance of the reference solution at 400 nm,

m_1 = mass of the mother tincture sample, in grams,

m_2 = mass of apigenin sample, in grams.

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