

## HORSE CHESTNUT (INFLORESCENCE) FOR HOMOEOPATHIC PREPARATIONS

### AESCULUS HIPPOCASTANUM FLOWERS FOR HOMOEOPATHIC PREPARATIONS

*Aesculi hippocastani flores ad praeparationes homoeopathicas*

#### DEFINITION

Fresh inflorescence of *Aesculus hippocastanum* L.

#### CHARACTERS

Macroscopic characters described under identification.

#### IDENTIFICATION

The inflorescence of horse chestnut, in pyramidal thyse is composed of white zygomorphous flowers often spotted with red or yellow. The flowers have a calyx with 5 deciduous, uneven teeth, a corolla with 5 petals, pubescent and uneven; the 3 lower petals are spread and bent, the 2 upper ones are erect and elliptical. The androecium contains 7 stamens with filaments arched outwardly, fused at the base into a nectariferous disk. The ovary, free with 3 biovular loculi, is topped by a single style ending by an acute stigma.

#### TESTS

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

**Loss on drying** (2.2.32): minimum 80.0 per cent, determined on 5.0 g of finely-cut drug, by drying in an oven at 105 °C for 2 h.

## STOCK

#### DEFINITION

The mother tincture of horse chestnut inflorescence complies with the requirements of the general technique for the preparation of the mother tincture (see *Homeopathic Preparations (1038)* and French Pharmacopoeia Supplement). The mother tincture is prepared with ethanol (55 per cent V/V) using the fresh inflorescence of *Aesculus hippocastanum* L.

*Content*: minimum 0.10 per cent *m/m* of total flavonoids, expressed as quercitrin (C<sub>21</sub>H<sub>20</sub>O<sub>11</sub>; M<sub>r</sub> 448.4).

---

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

## CHARACTERS

*Appearance:* brown liquid.

Characteristic odour.

## IDENTIFICATION

A. To 1 mL of mother tincture add 10 mL of *water R*. Shake. A persistent foam occurs (saponins).

B. Thin layer chromatography (2.2.27).

*Test solution.* Mother tincture.

*Reference solution.* Dissolve 5 mg of *kaempferol R*, 5 mg of *quercitrin R* and 5 mg of *isoquercitroside 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* (10:10:80 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 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                 |   |
|----------------------------------|---|
| Kaempferol: a green zone         | A green zone (kaempferol)<br>A blue zone                    |
| Quercitrin: an orange zone       | Two greenish-yellow zones<br>An orange zone (quercitrin)    |
| -----                            | -----   |
| Isoquercitroside: an orange zone | A greenish-yellow zone<br>An orange zone (isoquercitroside) |
| -----                            | -----   |
| A greenish-yellow zone           | A greenish-yellow zone (narcissin)                          |
| <b>Reference solution</b>        | <b>Test solution</b>  |

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

## TESTS

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

**Dry residue** (2.8.16): minimum 2.8 per cent *m/m*.

## ASSAY

Ultraviolet and visible spectrophotometry (2.2.25).

*Stock solution.* Evaporate 0.500 g of mother tincture to dryness, under reduced pressure. Dilute the residue in 25.0 mL of a mixture of 10 volumes of *methanol R* and 100 volumes of *glacial acetic acid R*.

*Test solution.* To 10.0 mL of stock 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* and dilute to 25.0 mL with *glacial acetic acid R*.

*Compensation liquid.* To 10.0 mL of mother tincture, add 10.0 mL of *anhydrous formic acid R* and dilute to 25.0 mL with *glacial acetic acid R*.

Thirty min later, measure the absorbance of the test solution at 415 nm, in comparison with the compensation liquid.

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

$$\frac{A \times 62.5}{400 \times m}$$

i.e. taking the specific absorbance of quercitrin to be 400.

*A* = absorbance of the test solution at 415 nm,

*m* = mass of the mother tincture sample, in grams.