

**HORSE CHESTNUT
FOR HOMOEOPATHIC PREPARATIONS
AESCULUS HIPPOCASTANUM
FOR HOMOEOPATHIC PREPARATIONS**

***Aesculus hippocastanum* (semen) ad praeparationes homoeopathicas**
Other Latin name used in homoeopathy: **Aesculus**

DEFINITION

Fresh seed of *Aesculus hippocastanum* L.

CHARACTERS

Macroscopic characters described in identification tests.

IDENTIFICATION

Horse chestnut seeds are more or less globose or ovoid. The outer integument is shiny, brown, and contains tannin. The large, whitish spot on the husk is the hilum.

The exalbuminous seed consists of two large fleshy, oily and amylaceous cotyledons which are often fused; the line of suture is more or less conspicuous. The curved radicle is situated in a depression either on the commissure or on the dorsal surface of one of the cotyledons.

TESTS

Foreign matter (2.8.2): maximum 2 per cent

Loss on drying (2.2.32): minimum 30.0 per cent, determined on 10.0 g of finely cut drug, by drying in an oven at 100-105 °C for 2 h.

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

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STOCK

DEFINITION

Horse chestnut mother tincture complies with the requirements of the general technique for the preparation of mother tinctures (see *Homoeopathic Preparations (1038)* and French Pharmacopoeia Authority Supplement). The mother tincture is prepared with ethanol (65 per cent *V/V*), using the fresh seed of *Aesculus hippocastanum* L.

Content: minimum 0.25 per cent *m/m* of total triterpene heterosides, expressed as aescin ($C_{55}H_{86}O_{24}$; M_r 1,131.3).

CHARACTERS

Yellow liquid.

IDENTIFICATION

A. Thin-layer chromatography (2.2.27).

Test solution. Mother tincture.

Reference solution. Dissolve 25 mg of aescin R and 2.5 mg of hederosaponin C R in ethanol (70 per cent *V/V*) R and dilute to 10 ml with the same solvent.

Plate: TLC silica gel plate R.

Mobile phase: water R, methanol R, glacial acetic acid R, methylene chloride R (2:3:8:15 *V/V/V/V*).

Application: 40 μ l, as bands.

Development: over a path of 12 cm

Drying: in air.

Detection: spray with anisaldehyde solution R and heat to 100-105 °C for 10 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.

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Top of the plate	
Aescin: two-three main greyish-blue zones Hederasaponin C: a greenish-brown zone	Two-three greyish-blue zones (aescin) Five greenish-brown zones
Reference solution	Test solution

TESTS

Ethanol (2.9.10): 60 per cent *V/V* to 70 per cent *V/V*.

Methanol and 2-propanol (2.9.11): maximum 0.05 per cent *V/V*; maximum 0,05 per cent *V/V*.

Dry residue: minimum 1.1 per cent *m/m* (see *French Pharmacopoeia Authority Supplement*).

ASSAY

Visible absorption spectrophotometry (2.2.25).

Test solution. Evaporate 5.00 g of mother tincture. Dissolve the residue in 20 ml of 0.1M hydrochloric acid and transfer to a separating funnel. Wash the flask with 2 quantities, each of 5 ml, of 0.1M hydrochloric acid. Combine the solutions and shake with a mixture of 20 ml of propanol R and 50 ml of methylene chloride R. Separate the lower organic phase. Carry out this extraction process twice. Combine the 3 organic phases in a 250 ml round-bottomed flask and evaporate to dryness in vacuo at 40-50°C. Wash the residue with 3 quantities, each of 10 ml, of ether R and discard the rinsing water. Evaporate the residual ether, then add 3 quantities, each of 10 ml, of glacial acetic acid R to the residue and wash the flask with a little glacial acetic acid R, filtering each solution through the filter used previously. Collect the filtrates in a 50.0 ml volumetric flask and dilute to the mark with the same solvent. To 1.0 ml of this solution, add 4.0 ml of ferric chloride-aceto-sulphuric solution R. Shake and heat for 25 min in a water-bath at 60°C, stirring occasionally. Cool rapidly to 20°C in running water for 5 min.

Reference solution. Dissolve 10.0 mg of aescin R in glacial acetic acid R and dilute to 50.0 ml with the same solvent. To 1.0 ml of this solution, add 4.0 ml of ferric chloride-aceto-sulphuric solution R. Shake and heat for 25 min on a water-bath at 60°C, stirring occasionally. Cool rapidly to 20°C in running water for 5 min.

Compensation liquid. To 1.0 ml of glacial acetic acid R add 4.0 ml of ferric chloride-aceto-sulphuric solution R. Shake and heat for 25 min on a water-

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bath at 60 °C, stirring occasionally. Cool rapidly to 20 °C in running water for 5 min.

Measure the absorbance of the test solution and the reference solution, by comparison with the compensation liquid at 540 nm.

Calculate the percentage content m/m of total triterpene heterosides, calculated as aescin, from the expression:

$$\frac{A_1 \times m_2 \times 100}{A_2 \times m_1}$$

A_1 = absorbance of the test solution,

A_2 = absorbance of the reference solution,

m_1 = mass of the mother tincture sample in milligrams,

m_2 = mass of aescin in the reference solution in milligrams.