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Horse Chestnut

DEFINITION

Horse Chestnut consists of the dried seeds of *Aesculus hippocastanum* L. (Fam. Hippocastanaceae) harvested in the fall. It contains NLT 3.0% of triterpene glycosides, calculated on the dried basis as escin ($C_{55}H_{86}O_{24}$).

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

Standard solution: 5 mg/mL of [USP Escin RS](#) in methanol

Sample solution: Transfer 1 g of the powdered plant material to a screw-capped centrifuge tube, add 10 mL of a mixture of alcohol and water (7:3), and heat on a steam bath for 10 min. Centrifuge, and use the clear supernatant.

Chromatographic system

(See [Chromatography \(621\)](#), [Thin-Layer Chromatography](#).)

Adsorbent: 0.25-mm layer of chromatographic silica gel (TLC plates)

Application volume: 10 µL

Developing solvent system: Use the upper phase of a mixture of 1-butanol, glacial acetic acid, and water (5:1:4).

Derivatization reagent: Methanol, glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (85:10:5:0.5)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatograms to a length of NLT 15 cm, and dry the plate in a stream of air. Spray the plate with *Derivatization reagent*, heat at 100° for 5 min, and examine under white light.

Acceptance criteria: The chromatogram of the *Sample solution* shows a blue-violet zone corresponding to escin, comparable in position and color to the main zone in the chromatogram of the *Standard solution*. Above this zone, the chromatogram of the *Sample solution* shows several narrow, brown to brownish-red zones that are less intense than the zone corresponding to escin.

COMPOSITION

• CONTENT OF TRITERPENE GLYCOSIDES

Solvent A: Methanol and water (13:7)

Solvent B: Use the lower phase of a mixture of chloroform, 0.1 N hydrochloric acid, and 1-propanol (5:3:2).

Reagent: Dissolve 75 mg of ferric chloride in 50 mL of ice-cold glacial acetic acid. Add 50 mL of sulfuric acid, while swirling on an ice bath. Prepare immediately before use.

Standard solution A: 0.2 mg/mL of [USP Escin RS](#) in glacial acetic acid, shaken for 1 min

Standard solution B: 0.4 mg/mL of [USP Escin RS](#) in glacial acetic acid, shaken for 1 min

Standard solution C: 0.6 mg/mL of [USP Escin RS](#) in glacial acetic acid, shaken for 1 min

Sample solution: Accurately weigh about 1 g of ground seeds, and transfer into a 250-mL round-bottom flask. Add exactly 100 mL of *Solvent A*, and weigh the filled flask with a precision of ±0.1 g. Attach a condenser, reflux for 30 min, and allow to cool. Adjust to the initial weight by adding *Solvent A*, and filter. Transfer 30.0 mL of the filtrate to a round-bottom flask, and evaporate under vacuum. Dissolve the residue in 20 mL of 0.1 N hydrochloric acid, and quantitatively transfer with the aid of two additional 5-mL portions of 0.1 N hydrochloric acid to a 250-mL separatory funnel. Add 20 mL of 1-propanol and 50 mL of chloroform, and shake vigorously for 2 min. Collect and retain the lower chloroform layer, and add 50 mL of *Solvent B* to the upper layer remaining in the separatory funnel. Shake vigorously for 2 min; collect and retain the lower chloroform layer. Combine the retained chloroform layers in a round-bottom flask, and evaporate to near-dryness under vacuum. Evaporate the remaining solvent under a stream of air. Wash the residue with two 10-mL aliquots of ether, filter, wash the filter with 10 mL of ether, and discard the ether filtrates. After evaporation of the residual ether, suspend the residue in 10 mL of glacial acetic acid, and pass through the previously used dried filter into a 50-mL volumetric flask. Repeat the addition of glacial acetic acid followed by filtration two additional times, combining the filtrates in the 50-mL volumetric flask. Wash the round-bottom flask with small quantities of glacial acetic acid, and filter into the volumetric flask. Dilute with glacial acetic acid to volume.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: Visible

Wavelength: 540 nm

Blank: Glacial acetic acid

Analysis: Accurately transfer 1.0 mL each of *Standard solutions A, B, and C*, *Sample solution*, and *Blank* in separate screw-cap test tubes.

Add 4.0 mL of *Reagent* to each tube, cap the tubes, and keep them on a water bath at 60° for 25 min, shaking occasionally, and measure the

absorbances of the reacted *Sample solution* and *Standard solutions A, B, and C*, corrected for the *Blank*. Plot the absorbances of *Standard solutions A, B, and C* against their respective concentrations, and establish the calibration line by linear regression. From the plot, determine the concentration, *C*, in mg/mL, of triterpene glycosides as escin in the *Sample solution*.

Calculate the percentage of triterpene glycosides as escin in the portion of Horse Chestnut taken:

$$\text{Result} = (C/W) \times (50/3)$$

C = concentration of triterpene glycosides in the *Sample solution* as obtained above (mg/mL)

W = weight of Horse Chestnut taken to prepare the *Sample solution* (g)

Acceptance criteria: NLT 3.0% of triterpene glycosides, calculated as escin ($C_{55}H_{86}O_{24}$), on the dried basis

CONTAMINANTS

- [ARTICLES OF BOTANICAL ORIGIN, Limits of Elemental Impurities \(561\)](#): Meets the requirements
- [ARTICLES OF BOTANICAL ORIGIN, Pesticide Residue Analysis \(561\)](#): Meets the requirements
- [MICROBIAL ENUMERATION TESTS \(2021\)](#): The total aerobic microbial count does not exceed 10^6 cfu/g, the total combined molds and yeast count does not exceed 10^4 cfu/g, and the bile-tolerant Gram-negative bacteria count is NMT 10^3 cfu/g.
- [ABSENCE OF SPECIFIED MICROORGANISMS \(2022\)](#): It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

SPECIFIC TESTS

BOTANICAL CHARACTERISTICS

Macroscopic: Horse chestnut seeds are dense and hard, subspherical to oval, slightly flattened, and from 2 to 4 cm in diameter. They have a dark brown seed coat from 1 to 1.5 mm thick, with a large, round, light brown spot (hilum). The seed coat is shiny, but only in fresh condition. The space under the coat is totally filled with the shiny, massive embryo and its large, pale yellow cotyledons lacking endosperm.

Microscopic: The epidermis of the testa in surface view has yellowish-brown cells of fairly uniform size, with the majority of cells rounded to polygonal, and a few that are square to obscurely triangular. The walls of these cells are considerably but rather unevenly thickened, and lack pits. In the sectional view, the cells are columnar, approximately 3–4 times as high as they are wide, with the outer periclinal wall markedly thickened, uneven, and becoming thinner toward the base; beneath the epidermis there are a few layers of small collenchymatously thickened cells with small intercellular spaces; the greater part of the testa consists of larger, loosely packed parenchymatous cells forming a spongy tissue; the walls are variably and unevenly thickened, with intercellular and large circular spaces well marked; the inner layer of the testa is a narrow zone, with ill-defined and thinner-walled cells. All the parenchymatous cells of the testa are darkly pigmented. The embryo has an outer layer of small colorless cells, almost square in sectional view, with outer and side walls thickened. In the surface view, only the irregular and more or less polygonal lumens are discernible, giving a reticulate, pitted appearance. Cotyledons are moderately thickened and indistinctly pitted, having round to ovoid parenchymatous cells densely filled with starch. Starch granules, mainly simple, are present in two size ranges: from 15 to 30 μm and from 3 to 10 μm . The largest granules vary from circular, ovoid, and bluntly polygonal to pyriform, most of them with a well-marked cleft or stellate hilum, and lacking striations. The smaller starch granules are less variable, spherical to ovoid, with the hilum more often a point. Compound starch granules are found very infrequently.

EXTRACTABLE MATTER

Analysis: Proceed as directed for [Articles of Botanical Origin \(561\)](#), [Alcohol-Soluble Extractives, Method 2](#), except use a mixture of methanol and water (8:2) instead of alcohol.

Acceptance criteria: NLT 18.0%

- [ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter \(561\)](#): NMT 2.0%
- [LOSS ON DRYING \(731\)](#): Dry a sample at 105° for 2 h: it loses NMT 10.0% of its weight.
- [ARTICLES OF BOTANICAL ORIGIN, Total Ash \(561\)](#): NMT 4.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in a well-closed, light-resistant container, protected from moisture.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant contained in the article.
- [USP REFERENCE STANDARDS \(11\)](#)
[USP Escin RS](#)

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
HORSE CHESTNUT	Anton Bzhelyansky Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

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