

Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-Jan-2018
Document Type: Dietary Supplement Monographs
DocId: GUID-75DD4CA1-9435-4D5E-BE53-D009EFFDC1A6_3_en-US
DOI: https://doi.org/10.31003/USPNF_M14924_03_01
DOI Ref: 9p8kf

© 2025 USPC
Do not distribute

Powdered Horse Chestnut Extract

DEFINITION

Powdered Horse Chestnut Extract is prepared from Horse Chestnut by extraction with alcohol–water mixtures or methanol–water mixtures.

The ratio of starting plant material to extract is between 5:1 and 8:1. It contains NLT 90.0% and NMT 110.0% of the labeled amount of triterpene glycosides, calculated on the dried basis as escin ($C_{55}H_{86}O_{24}$). It may contain suitable added substances.

IDENTIFICATION

• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

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

Sample solution: To 10 mL of methanol add a quantity of Powdered Extract equivalent to 25 mg of the labeled amount of triterpene glycosides, and shake. Allow to stand for 15 min before use.

Chromatographic system

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

Adsorbent: 0.25-mm layer of chromatographic silica gel

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).

Spray 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 current of air. Spray the plate with *Spray reagent*, heat the plate at 100° for 5 min, and examine the plate under daylight.

Acceptance criteria: The chromatogram from the *Sample solution* shows a blue-violet zone corresponding to escin, comparable in position and color to the main zone in the chromatogram from 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 glacial acetic acid. Add 50 mL of sulfuric acid, while shaking and cooling. Prepare immediately before use.

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

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

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

Sample solution: Transfer a quantity of Powdered Extract, equivalent to 50 mg of the labeled content of triterpene glycosides, into a 50-mL flask. Add 20 mL of 0.1 N hydrochloric acid, and shake for 5 min. Filter into a 250-mL separatory funnel with the aid of two additional 5-mL portions of 0.1 N hydrochloric acid. Add 20 mL of 1-propanol and 50 mL of chloroform, and shake vigorously for 2 min. Separate the chloroform layer, and add *Solvent B* to the upper phase remaining in the separation funnel. Shake vigorously for 2 min, and separate the chloroform layer. Combine the chloroform layers in a round-bottom flask, and evaporate to dryness under vacuum. Evaporate the remaining solvents with the aid of a current of air. Wash the residue with two 10-mL portions of ether, filter, wash the filter with 10 mL of ether, and discard the ether filtrates. After evaporation of the residual ether, add to the residue a 10-mL portion of glacial acetic acid, and pass through the previously used dried filter into a 100-mL volumetric flask. Repeat the addition of glacial acetic acid followed by filtration two additional times, combining the filtrates in the 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: Transfer 1 mL each of *Standard solutions A, B, and C*, the *Sample solution*, and the *Blank* to separate test tubes with stoppers. Add 4.0 mL of *Reagent* to each tube, cap the tubes, and place them in a water bath at 60° for 25 min, shaking occasionally. Measure the absorbances of the reacted *Sample solution* and the reacted *Standard solutions A, B, and C*, and correct for the *Blank*. Plot the absorbances

of the reacted *Standard solutions A, B, and C* versus concentrations, in mg/mL of [USP Escin RS](#) in the corresponding *Standard solution*. From the graphs determine the concentration, *C*, in mg/mL, of triterpene glycosides as escin ($C_{55}H_{86}O_{24}$) in the *Sample solution*.

Calculate the percentage of the labeled amount of triterpene glycosides in the portion of Powdered Extract taken:

$$\text{Result} = (C/C_U) \times 100$$

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

C_U = nominal concentration of triterpene glycosides in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of triterpene glycosides as escin ($C_{55}H_{86}O_{24}$) on the dried basis

CONTAMINANTS

- **MICROBIAL ENUMERATION TESTS (2021):** The total aerobic microbial count does not exceed 10^4 cfu/g, the total combined molds and yeasts count does not exceed 10^2 cfu/g, and the count for enterobacteria does not exceed 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

- **LOSS ON DRYING (731):** Dry 1 g at 105° for 2 h: it loses NMT 5.0% of its weight.
- **OTHER REQUIREMENTS:** It meets the requirements in [Botanical Extracts \(565\)](#), [General Pharmacopeial Requirements](#), for *Packaging and Storage*, *Residual Solvents*, and *Pesticide Residues* for powdered extracts.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cool place.
- **LABELING:** The label states the Latin binomial and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of triterpene glycosides, the extracting solvent or solvent mixture used for preparation, the ratio of the starting crude plant material to Powdered Extract, and the name and content of any added substance. It meets the requirements for labeling in [Botanical Extracts \(565\)](#).
- **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
POWDERED HORSE CHESTNUT EXTRACT	Anton Bzhelyansky Scientific Liaison	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BDSHM2020 Botanical Dietary Supplements and Herbal Medicines

Chromatographic Database Information: [Chromatographic Database](#)

Most Recently Appeared In:

Pharmacopeial Forum: Volume No. PF 30(2)

Current DocID: [GUID-75DD4CA1-9435-4D5E-BE53-D009EFFDC1A6_3_en-US](#)

Previous DocID: [GUID-75DD4CA1-9435-4D5E-BE53-D009EFFDC1A6_1_en-US](#)

DOI: https://doi.org/10.31003/USPNF_M14924_03_01

DOI ref: [9p8kf](#)