Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-May-2024
Document Type: USP Monographs
DocId: GUID-C4D2B4D7-ADD2-41C0-88D8-51C464139840_2_en-US
DOI: https://doi.org/10.31003/USPNF_M10759_02_01
DOI Ref: avj3r

Add the following:

▲*Picrorhiza* Species Root and Rhizome

DEFINITION

Picrorhiza Species Root and Rhizome consists of the dried root and rhizome of *Picrorhiza kurroa* Royle ex Benth. (Fam. Plantaginaceae).[1]

Picrorhiza Species Root and Rhizome contains NLT 3.5% of iridoid glycosides, calculated as the sum of picroside I and picroside II, on the anhydrous basis.

IDENTIFICATION

• **A. HPTLC for Articles of Botanical Origin ⟨203⟩**

Standard solution A: 0.5 mg/mL of USP Picroside I RS in methanol

Standard solution B: 80 mg/mL of USP *Picrorhiza kurroa* Root and Rhizome Dry Extract RS in methanol

Sample solution: Sonicate 500 mg of *Picrorhiza* Species Root and Rhizome, finely powdered and accurately weighed, in 5 mL of methanol for 15 min. Centrifuge and use the supernatant.

Chromatographic system

(See standard parameters as defined in *HPTLC for Articles of Botanical Origin ⟨203⟩*, *Table 1*.)

Application volume: 2 µL each of *Standard solution A*, *Standard solution B*, and the *Sample solution*, as 8-mm bands

Developing solvent system: 2-Butanone, isopropyl alcohol, and formic acid (90:10:5)

Derivatization reagent: Prepare the anisaldehyde–sulfuric acid reagent as follows. Add 20 mL of acetic acid and 10 mL of sulfuric acid to 170 mL of cold methanol and mix well. After cooling to room temperature, add 1 mL of anisaldehyde.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable HPTLC plate and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry. Treat with the *Derivatization reagent*, heat for 3 min at 100°, and examine under long-wave UV light and under white light.

System suitability

Under long-wave UV light: *Standard solution B* exhibits about 5–6 blue or purple bands and/or yellow, brown, or green bands in the lower-half section, with the band near the middle section (near R_F 0.45) corresponding to picroside I in *Standard solution A*. The intense blue fluorescent band at about R_F 0.25 corresponds to picroside II. Additional blue or purple and/or pink or green fluorescent bands are observed in the upper-half section.

Under white light: *Standard solution B* exhibits about 4–5 brown bands in the lower-half section, with the most intense band close to the upper-most section (near R_F 0.45) and corresponding in R_F and color to picroside I in *Standard solution A*. Another intense band (near R_F 0.25) corresponds to picroside II.

Acceptance criteria

Under long-wave UV light: The *Sample solution* exhibits a blue or purple fluorescent band and a yellow, brown, or green fluorescent band in the lower-half section (near R_F 0.45) corresponding in R_F and color to picroside I in *Standard solution A*. The most intense blue fluorescent band (near R_F 0.25) corresponds to picroside II. Additional blue or purple and/or pink or green fluorescent bands are observed in the upper-half section.

Under white light: The *Sample solution* exhibits about 4–5 brown bands in the lower-half section, with the most intense band close to the upper-most section (near R_F 0.45) and corresponding in R_F and color to picroside I in *Standard solution A*. Another intense band (near R_F 0.25) corresponds to picroside II.

• **B. HPLC**

Analysis: Proceed as directed in the test for *Content of Iridoid Glycosides*.

Acceptance criteria: The *Sample solution* exhibits the most intense peak at a retention time corresponding to picroside I in *Standard solution A* and *Standard solution B*. The second most intense peak is picroside II. Three additional peaks, two between picroside I and picroside II and one before picroside II, are observed.

COMPOSITION

• **Content of Iridoid Glycosides**

Solution A: Dissolve 0.136 g of anhydrous potassium phosphate, monobasic in 900 mL of water, and add 0.5 mL of phosphoric acid. Dilute with water to 1000 mL.

Solution B: Acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
7	80	20
15	70	30
20	20	80
25	85	15
30	85	15

Standard solution A: 0.015 mg/mL of USP Picroside I RS in methanol

Standard solution B: 0.6 mg/mL of USP *Picrorhiza kurroa* Root and Rhizome Dry Extract RS in methanol. Sonicate if necessary. Before injection, pass the solution through a suitable membrane filter of 0.45-µm or finer pore size. Discard the first few milliliters of the filtrate.

Sample solution: Transfer 0.5 g of *Picrorhiza* Species Root and Rhizome, finely powdered and accurately weighed, to a suitable flask. Add 50 mL of methanol and reflux for 20 min. Repeat 4–5 times for exhaustive extraction. Combine each extract and dilute with methanol to 100 mL. Before injection, pass the solution through a suitable membrane filter of 0.45-µm or finer pore size. Discard the first few milliliters of the filtrate.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 263 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solution A* and *Standard solution B*

Suitability requirements

Resolution: NLT 1.5 between picroside I and the peak before, *Standard solution B*

Tailing factor: NMT 1.5 for picroside I, *Standard solution A*

Relative standard deviation: NMT 2.5% for picroside I in repeated injections, *Standard solution A*

Chromatogram similarity: The chromatogram of *Standard solution B* is similar to the reference chromatogram provided with the lot of USP *Picrorhiza kurroa* Root and Rhizome Dry Extract RS being used.

Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Using the chromatograms of *Standard solution A* and *Standard solution B* and the reference chromatogram provided with the lot of USP *Picrorhiza kurroa* Root and Rhizome Dry Extract RS being used, identify the retention times of the peaks corresponding to picroside I and picroside II. The approximate relative retention times of the peaks for picroside I and picroside II are 1.00 and 0.77, respectively.

Calculate separately the percentages of picroside I and picroside II in the portion of *Picrorhiza* Species Root and Rhizome taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times F \times 100$$

r_U = peak area of picroside I or picroside II from the *Sample solution*

r_S = peak area of picroside I from *Standard solution A*

C_S = concentration of USP Picroside I RS in *Standard solution A* (mg/mL)

V = volume of the *Sample solution* (mL)

W = weight of *Picrorhiza* Species Root and Rhizome used to prepare the *Sample solution* (mg)

F = conversion factor for the analyte (1.0 for picroside I, 1.74 for picroside II)

Calculate the content of iridoid glycosides as the sum of the percentages of picroside I and picroside II.

Acceptance criteria: NLT 3.5% on the anhydrous basis

CONTAMINANTS

• **Articles of Botanical Origin ⟨561⟩, *Limits of Elemental Impurities*:** Meets the requirements

• **Articles of Botanical Origin ⟨561⟩, *Pesticide Residue Analysis*:** Meets the requirements

• **Microbial Enumeration Tests ⟨2021⟩:** The total aerobic bacterial count does not exceed 10^5 cfu/g, the total combined molds and yeasts count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacterial count does not exceed 10^3 cfu/g.

• **Absence of Specified Microorganisms ⟨2022⟩, *Test Procedures, Test for Absence of Salmonella Species*** and ***Test for Absence of Escherichia coli*:** Meets the requirements

SPECIFIC TESTS

• **Botanical Characteristics**

Macroscopic

Rhizome: 2.5–12.0 cm long and 0.3–1.0 cm thick; subcylindrical, straight or slightly curved; externally grayish-brown; surface rough due to longitudinal wrinkles; circular scars of roots and bud scales, sometimes roots attached, tip ends in a growing bud surrounded by a tufted crown of leaves; in-place cork exfoliates exposing dark cortex; fracture short.

Root: Thin, cylindrical, 5–10 cm long and 0.5–1.0 mm in diameter; straight or slightly curved with a few longitudinal wrinkles and dotted scars, mostly attached with rhizomes; dusty gray; fracture short; inner surface black with whitish xylem.

Microscopic

Rhizome: 20–25 layers of cork consisting of tangentially elongated, suberized cells; cork cambium is 1–2 layers; cortex is single-layered or absent, primary cortex persists in some cases, 1–2 small vascular bundles are present in the cortex. Vascular bundles are surrounded by fibrous bundle sheath. Secondary phloem is composed of parenchyma cells and a few scattered fibers. Cambium is 2–4 layered. Secondary xylem consists of vessels, tracheids, fibers, and parenchyma cells. Vessels vary in size and shape, having transverse oblique articulation; tracheids are long, thick-walled, lignified, and more or less cylindrical with blunt, tapering ends. Starch grains are abundant, 25–105 µm in diameter.

Root: Young roots show single-layered epidermis, some epidermal cells elongate forming unicellular hairs. Hypodermis is single-layered. Cortex 8–14 is layered, consisting of oval to polygonal, thick-walled parenchymatous cells. Primary stele, tetrarch to heptarch, is enclosed by a single-layered pericycle and single-layered thick-walled endodermis cells. Mature roots show 4–15 layers of cork with 1–2 layers of cork cambium. Vessels vary in size and shape—some are cylindrical with tail-like, tapering ends; some are drum shaped with perforation on lateral or end walls. Tracheids are cylindrical with tapering pointed ends.

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Foreign Organic Matter*:** NMT 2.0%

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Alcohol-Soluble Extractives, Method 1*:** NLT 10.0%

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Water-Soluble Extractives, Method 1*:** NLT 20.0%

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Total Ash*:** NMT 7.0%

• **Articles of Botanical Origin ⟨561⟩, *Methods of Analysis, Acid-Insoluble Ash*:** NMT 1.0%

• **Water Determination ⟨921⟩, *Method III***

Sample: 2.0 g of *Picrorhiza* Species Root and Rhizome, finely powdered

Analysis: Dry the *Sample* at 105° for 2 h.

Acceptance criteria: NMT 10%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at controlled room temperature.

• **Labeling:** The label states the Latin binomial following the official name of the plant from which the article was derived.

• **USP Reference Standards ⟨11⟩**

USP *Picrorhiza kurroa* Root and Rhizome Dry Extract RS

USP Picroside I RS▲ (USP 1-May-2024)

[1] The synonym status of the species names *Picrorhiza kurroa* Royle ex Benth. and *Picrorhiza kurrooa* Royle is unresolved at this time. Previously, this species was placed in the Family Scrophulariaceae.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
PICRORHIZA SPECIES ROOT AND RHIZOME	Nam-Cheol Kim 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. 48(5)

Current DocID: GUID-C4D2B4D7-ADD2-41C0-88D8-51C464139840_2_en-US
DOI: https://doi.org/10.31003/USPNF_M10759_02_01
DOI ref: avj3r