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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*