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Indian Barberrry Stem

DEFINITION

Indian Barberrry Stem consists of the stems of *Berberis aristata* DC. (Family Berberidaceae). It contains NLT 1.0% of berberine, calculated on the dried basis.

IDENTIFICATION

• **A. HPTLC FOR ARTICLES OF BOTANICAL ORIGIN (203).**

Standard solution A: 1 mg of [USP Berberine Chloride RS](#) in 8 mL of [methanol](#) and 1 mg of [USP Palmatine Chloride RS](#) in 16 mL of [methanol](#)

Standard solution B: Sonicate 50 mg of [USP Berberis aristata Stem Dry Extract RS](#) in 4 mL of 80% [methanol](#) for 10 min. Centrifuge or filter, and use the supernatant or the filtrate.

Sample solution: Transfer 0.25 g of Indian Barberrry Stem, finely powdered, to a beaker and add 4 mL of 80% [methanol](#). Sonicate for 10 min, centrifuge or filter, and use the supernatant.

Chromatographic system

Adsorbent: [Chromatographic silica gel F₂₅₄ mixture](#) with an average particle size of 5 µm

Application volume: 10 µL each of *Standard solution A*, *Standard solution B*, and *Sample solution* as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of about 33% using a suitable device.

Temperature: About 25°

Developing solvent system: [Ethyl acetate](#), [formic acid](#), and [water](#) (80:10:10)

Developing distance: 7 cm

Derivatization reagent: 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](#) to the mixture.

Analysis

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

Apply the *Samples* as bands and dry in air. Develop in a saturated chamber, remove the plate from the chamber, and dry in air. Examine under UV light at 254 and 365 nm before derivatization. Then treat with *Derivatization reagent*, and examine under white light.

System suitability

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

Suitability requirements: Under UV 254 nm light, *Standard solution B* exhibits one gray band near the middle of the chromatogram, slightly overlapping with a pale red band, at an R_f corresponding to the berberine band from *Standard solution A*. Two additional pale gray bands appear below the berberine band; the lower band corresponds in R_f to the palmatine band from *Standard solution A*. Under UV 365 nm light, *Standard solution B* exhibits one intense yellow band near the middle of the chromatogram at an R_f corresponding to berberine. A pale yellow band appears below the berberine band, and another yellow band, less intense than the band due to berberine, appears beneath it at an R_f corresponding to palmatine. Under white light after derivatization, *Standard solution B* exhibits one yellow band near the middle of the chromatogram at an R_f corresponding to berberine.

Acceptance criteria: Under UV 254 nm light, the *Sample solution* exhibits a gray band near the middle of the chromatogram, slightly overlapping with a pale red band, at an R_f corresponding to berberine. Another gray band that slightly overlaps with another pale red band appears below the berberine band at an R_f corresponding to palmatine in *Standard solution A*. A pale gray band appears between the berberine and palmatine bands. Under UV 365 nm light, the *Sample solution* exhibits two intense yellow bands near the middle of the chromatogram; the higher R_f corresponds to berberine and the lower R_f corresponds to palmatine in *Standard solution A*. A pale yellow band appears between the berberine and palmatine bands. A number of pale yellow and pale blue bands overlap below the palmatine band. Under white light after derivatization, the *Sample solution* exhibits two yellow bands near the middle of the chromatogram; the higher R_f corresponds to berberine and the lower R_f corresponds to palmatine in *Standard solution A*.

• **B. HPLC**

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

Acceptance criteria: The chromatogram of the *Sample solution* exhibits peaks at retention times corresponding to the peaks due to berberine in *Standard solution B*.

COMPOSITION

• CONTENT OF BERBERINE

Solution A: Transfer 0.6 g of [sodium 1-hexanesulfonate](#) into a 1-L volumetric flask, and add 900 mL of [water](#) to dissolve. Adjust with [glacial acetic acid](#) to a pH of 2.7, and dilute with [water](#) to volume.

Solution B: [Acetonitrile](#)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	70	30
18	70	30
22	20	80
26	20	80
30	90	10
35	90	10

Standard solution A: 0.1 mg/mL of [USP Berberine Chloride RS](#) in [methanol](#)

Standard solution B: Transfer [USP Berberis aristata Stem Dry Extract RS](#), equivalent to 5 mg of berberine, into a 50-mL volumetric flask, and add 25 mL of [methanol](#). Heat at 80° with sonication for 20 min. Cool the solution and dilute with [methanol](#) to volume. Pass through a membrane filter of 0.45-µm or finer pore size, and use the filtrate.

Sample solution: Transfer 0.5 g of Indian Barberry Stem, finely powdered and accurately weighed, into a 100-mL centrifuge tube, and add 50 mL of [methanol](#). Sonicate for 5 min, centrifuge, and transfer the supernatant to a 100-mL volumetric flask. Repeat the extraction two more times by adding 20 mL of [methanol](#) and transferring the supernatant to the same 100-mL volumetric flask. Dilute with [methanol](#) to volume.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 348 nm

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

Column temperature: 25°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.7 between the berberine peak and the previous peak, *Standard solution B*

Tailing factor: NMT 2.0 for the berberine peak, *Standard solution A*

Relative standard deviation: NMT 2.0% for the berberine peak 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 Berberis aristata Stem Dry Extract RS](#) being used.

Analysis

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

Using the chromatograms of *Standard solution A*, *Standard solution B*, and the reference chromatogram provided with the lot of [USP Berberis aristata Stem Dry Extract RS](#) being used, identify the retention times of the peaks corresponding to berberine in the *Sample solution*.

Calculate the percentage of berberine in the portion of Indian Barberry Stem taken:

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

r_U = peak area of berberine from the *Sample solution*

r_S = peak area of berberine from *Standard solution A*

C_S = concentration of [USP Berberine Chloride RS](#) in *Standard solution A* (mg/mL)

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