

Status: Currently Official on 16-Feb-2025
 Official Date: Official as of 01-Feb-2019
 Document Type: Dietary Supplement Monographs
 DocId: GUID-73FB679C-E2E1-489E-8F2B-24062AEEF576_5_en-US
 DOI: https://doi.org/10.31003/USPNF_M9818_05_01
 DOI Ref: 3yk8g

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Change to read:

Salix Species Bark Powder

▲ (ERR 1-Feb-2019)

DEFINITION

Salix Species Bark Powder consists of Salix Species Bark reduced to fine or very fine powder. It contains NLT 1.50% of total salicylate derivatives, calculated as salicin ($C_{13}H_{18}O_7$) on the dried basis. Salix Species Bark Powder contains NMT 1.50% of free salicin, calculated on the dried basis.

IDENTIFICATION

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

Standard solution A: 1.50 mg/mL of [USP Salicin RS](#) in [methanol](#)

Standard solution B: 30 mg/mL of [USP Salix Species Bark Dry Extract RS](#) in [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution A: Suspend 1000 mg of Salix Species Bark Powder in 10.0 mL of [methanol](#). Sonicate for 10 min, centrifuge, and use the supernatant.

Sample solution B: Combine 5.0 mL of Sample solution A with 1.0 mL of 50-mg/mL [anhydrous sodium carbonate](#). Cap tightly and incubate at 60° for 10 min. Centrifuge and use the supernatant.

Chromatographic system

Adsorbent: Chromatographic silica gel with an average particle size of 5 µm (HPTLC plate)¹

Application volume: 5.0 µL each of Standard solution A, Standard solution B, and Sample solution A and 6.0 µL of Sample solution B as 8-mm bands

Relative humidity: Condition the plate to a relative humidity of 33%.

Temperature: Ambient, not to exceed 30°

Developing solvent system: [Ethyl acetate](#), [methanol](#), and [water](#) (77:13:10)

Developing distance: 6 cm

Derivatization reagent: [Sulfuric acid](#) and [methanol](#) (1:9). Slowly add sulfuric acid to ice-cold methanol.

Analysis

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

Apply the Samples as bands and dry in air. Develop in a saturated chamber, and dry in a current of air for 5 min. Treat with Derivatization reagent, heat at 100° for 5 min, and examine under white light.

System suitability: Under white light, the derivatized chromatogram of Standard solution B displays, in the middle third of the plate, three brown bands: the lower corresponds to the salicin band in Standard solution A; the one above it is due to salicortin; the top band is due to tremulacin. A faint band, which may appear between the salicortin and tremulacin bands, is due to tremuloidin. Two darker brown bands are seen in the lower third of the plate: one proximate to the application line; another, more intense, above it.

Acceptance criteria: Under white light, the derivatized chromatogram of Sample solution A shows one or several dark bands due to different salicin esters, whose position and intensity are contingent on the Salix species being used. The salicylate bands of interest are predominantly located in the middle third of the plate, demarcated by the salicin and tremulacin bands of Standard solution B. The salicin band in Sample solution A, corresponding to that in Standard solution A, may be faint or not visible. Additional bands may be seen in Sample solution A and Sample solution B. In Sample solution B, the bands due to salicin esters are not present, while the salicin band corresponding to that in Standard solution A is the principal band observed. The salicin band in Sample solution A is of lower intensity than the corresponding band in Standard solution A. The salicin band in Sample solution B is of comparable or higher intensity than the corresponding band in Standard solution A.

• **B. HPLC**

Analysis: Proceed as directed in the test for Salicylates Profile and Limit of Free Salicin.

Acceptance criteria: The *Sample solution* exhibits peaks at retention times corresponding to those of salicin and salicin derivatives in the Standard solutions.

COMPOSITION

• CONTENT OF SALICIN

Diluent: [Methanol](#) and [water](#) (1:1)

Solution A: 0.01% [trifluoroacetic acid](#)

Solution B: [Acetonitrile](#)

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

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	85	15
30	50	50
32	10	90
35	10	90
37	90	10
45	90	10

Standard solution A: 0.50 mg/mL of [USP Salicin RS](#) in *Diluent*

Sample solution: Accurately weigh about 1.3 g of *Salix* Species Bark Powder, transfer to a 200-mL round-bottom flask, and add 40 mL of [methanol](#) and 3 mL of [1 N sodium hydroxide](#). Attach the condenser and heat under reflux for 2 h, with intermittent shaking. Allow to cool, neutralize with 3 mL of [1 N hydrochloric acid](#), and pass through a paper filter into a 100-mL volumetric flask. Wash the round-bottom flask twice with 5-mL aliquots of [methanol](#) and filter into the same 100-mL volumetric flask. Adjust with [water](#) to volume, mix well, allow to equilibrate to room temperature, and readjust with water. Pass through a PTFE filter of 0.45-µm pore size, discarding the initial 3 mL of the filtrate.

Chromatographic system

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

Mode: LC

Detector: UV 270 nm

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

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution A*

Suitability requirements

Tailing factor: 0.8–2.0 for the salicin peak

Relative standard deviation: NMT 2.0% for the salicin peak in replicate injections

Analysis

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

Using the chromatogram of *Standard solution A*, identify the salicin peak in the *Sample solution* chromatogram.

Calculate the percentage of salicin hydrolytically derived from constituent salicylates in the portion of *Salix* Species Bark Powder taken:

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

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