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## Salix Species Bark

▲ (ERR 1-Feb-2019)

### DEFINITION

*Salix* Species Bark is prepared from the whole or fragmented dried bark of the young branches, or whole dried pieces of the current-year twigs, obtained from *Salix* species (Fam. Salicaceae). Common in pharmacopeial use are *S. alba* L., *S. babylonica* L., *S. daphnoides* Vill., *S. fragilis* L., *S. chilensis* Molina, *S. pentandra* L., *S. purpurea* L., and a number of other complying willow species and their hybrids. It contains NLT 1.50% of total salicylate derivatives, calculated as salicin ( $C_{13}H_{18}O_7$ ) 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, finely powdered, 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)<sup>1</sup>

**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*.

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

## 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<br>(min) | Solution A<br>(%) | Solution B<br>(%) |
|---------------|-------------------|-------------------|
| 0             | 90                | 10                |
| 10            | 85                | 15                |
| 30            | 50                | 50                |
| 32            | 10                | 90                |
| 35            | 10                | 90                |
| 37            | 90                | 10                |
| 45            | 90                | 10                |

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

**Sample solution:** Reduce to a fine powder and accurately weigh about 1.3 g of *Salix Species Bark*, 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*

#### 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* and *Sample solution*

Using the chromatogram of the *Standard solution*, 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* 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*