Salix Species Bark Family of monographs

Type of Posting	Notice of Intent to Revise
Posting Date	25–Aug–2017
Official Date	01–Dec–2017, Revision Bulletin and 01–Mar–2018, Interim Revision Announcement
Expert Committee	Botanical Dietary Supplements and Herbal Medicines

In accordance with section 7.04 (c) of the 2015–2020 Rules and Procedures of the Council of Experts, this is to provide notice that the Botanical Dietary Supplements and Herbal Medicines Expert Committee intends to revise the *Salix* Species family of monographs, including *Salix* Species Bark, *Salix* Species Bark Powder, and *Salix* Species Bark Dry Extract.

The Salix monographs were initially published for comment in *Pharmacopeial Forum* 42(1) [Jan.–Feb. 2016] and subsequently approved for inclusion in the *Second Supplement* to *USP 40–NF 35*, which will become official on December 1, 2017. Based on safety concerns the Botanical Dietary Supplements and Herbal Medicines Expert Committee has recommended the addition of the following labeling statement to the Salix Species family of monographs which were approved for publication in the *Second Supplement* to *USP 40–NF 35*: "**Dosage forms prepared with this article should bear the following statement: Not for use in children, women who are pregnant or nursing, or by persons with known sensitivity to aspirin**."

It is anticipated that proposed Interim Revisions Announcements (IRAs) to the Salix species monographs will be published in *Pharmacopeial Forum* 43(5) [Sep.–Oct. 2017] pursuant to section 7.02 of the Rules and Procedures. The comment period for these revisions will end on November 30, 2017. Unless otherwise decided by the Botanical Dietary Supplements and Herbal Medicines Expert Committee based on comments received, the IRAs will be posted online on January 26, 2018 and will become official on March 1, 2018.

In addition, it is also anticipated that the Botanical Dietary Supplements and Herbal Medicines Expert Committee will postpone the official date of the Salix Species monographs via Revision Bulletins to be posted July 28, 2017 and become official December 1, 2017. The postponements will remain in effect until the IRAs to be published in *Pharmacopeial Forum* 43(5) become official.

Should you have any questions, please contact Anton Bzhelyansky, Scientific Liaison to the Botanical Dietary Supplements and Herbal Medicines Expert Committee at (301-230-6303 or anb@usp.org).

Change to read:

■*Salix* Species Bark

•(This monograph is postponed indefinitely.)• (RB 1-Dec-2017)

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)[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*.

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 (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: 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

[1] A suitable commercially available plate is HPTLC Silica Gel 60 F_{254} from EMD Millipore (e.g., Part No. 1.05642.0001).

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*
r_S = peak area of salicin from the *Standard solution*
C_S = concentration of USP Salicin RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution* (mL)
W = weight of *Salix* Species Bark taken to prepare the *Sample solution* (mg)

Acceptance criteria: NLT 1.50% of salicin on the dried 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 yeasts and molds count does not exceed 10^3 cfu/g, and the bile-tolerant Gram-negative bacteria 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

- **Salicylates Profile**
  Diluent, Mobile phase, and **Chromatographic system:** Proceed as directed in the test for *Content of Salicin*.
  Standard solution: 5 mg/mL of USP *Salix* Species Bark Dry Extract RS in *Diluent*. Sonicate for 5 min, mix well, and pass through a PTFE filter of 0.45-µm pore size, discarding the initial 3 mL of the filtrate.
  Sample solution: Reduce to fine powder and accurately weigh about 650 mg of *Salix* Species Bark, transfer to a 50-mL volumetric flask, add 25 mL of methanol, and sonicate for 30 min. 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.
  System suitability
  Suitability requirements: The chromatogram of the *Standard solution* is similar to the reference chromatogram provided with the lot of USP *Salix* Species Bark Dry Extract RS being used.
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Using the chromatogram of the *Standard solution* and the reference chromatogram provided with the lot of USP *Salix* Species Bark Dry Extract RS being used, identify salicin esters present in the *Sample solution B* chromatogram. The approximate relative retention times, with respect to salicin, are provided in *Table 2*.

Table 2

Analyte	Relative Retention Time
Salicin	1.0
Salicortin	3.0
Tremuloidin	3.6
Tremulacin	4.6

  Acceptance criteria: The peak area of salicin is NMT 50% of the combined peak areas of all identified constituent salicylates.
- **Botanical Characteristics**
  Macroscopic: The bark is 1–2 cm wide and 1–2 mm thick, and occurs in flexible, elongated, quilled or curved pieces. The outer surface is glossy, smooth or slightly wrinkled longitudinally; greenish yellow in the younger bark to brownish grey in the older bark. The inner surface is smooth or finely striated longitudinally and white, pale yellow or reddish brown, depending on the species. The fracture is short in the outer part and coarsely fibrous in the inner region, and is easily split longitudinally. The diameter of current year twigs is NMT 10 mm. The xylem of young twigs is white or pale yellow.
  Microscopic: Two or three rows of poorly developed cork cells with thickened outer walls; cortex of collenchymatous and parenchymatous cells. The latter contains cluster crystals of calcium oxalate, 20–25 µm in diameter, and occasionally tannin. Phloem is characterized by tangential groups of lignified fibers associated with a crystal sheath containing prismatic crystals of calcium oxalate. Simple, rounded starch granules 6–8 µm in diameter in the parenchymatous cells of the phloem and medullary rays.
- **Articles of Botanical Origin** ⟨**561**⟩**,** *Methods of Analysis, Foreign Organic Matter***:** NMT 3.0% of twigs with a diameter greater than 10 mm, and NMT 2.0% of other foreign matter
- **Loss on Drying** ⟨**731**⟩
  Sample: 1.0 g of *Salix* Species Bark, finely powdered
  Analysis: Dry the *Sample* at 105° for 2 h.
  Acceptance criteria: NMT 10.0%
- **Articles of Botanical Origin** ⟨**561**⟩**,** *Methods of Analysis, Total Ash*
  Sample: 2.0 g of *Salix* Species Bark, finely powdered
  Acceptance criteria: NMT 10.0%
- **Articles of Botanical Origin** ⟨**561**⟩**,** *Methods of Analysis, Acid-Insoluble Ash*
  Sample: 2.0 g of *Salix* Species Bark, finely powdered
  Acceptance criteria: NMT 3.0%
- **Articles of Botanical Origin** ⟨**561**⟩**,** *Methods of Analysis, Water-Soluble Extractives*
  Sample: 2.0 g of *Salix* Species Bark, finely powdered
  Acceptance criteria: NLT 10.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers, protected from light and moisture, and store at room temperature.
- **Labeling:** The label states the Latin binomial(s) of one or several *Salix* species included in the article.
- **USP Reference Standards** ⟨**11**⟩
  USP Salicin RS
  USP *Salix* Species Bark Dry Extract RS

●(This monograph is postponed indefinitely.)● (RB 1-Dec-2017)

■2S (*USP40*)