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 Dry Extract

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

DEFINITION

Salix Species Bark Dry Extract is prepared from *Salix* Species Bark by extraction with hydroalcoholic, aqueous, or other suitable solvents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of salicylates, calculated as salicin ($C_{13}H_{18}O_7$) on the anhydrous basis. The ratio of starting plant material to extract is between 5:1 and 20:1. It may contain suitable added substances.

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 the amount of *Salix* Species Bark Dry Extract calculated to contain 15 mg of salicin (post-hydrolysis) 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*.

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

• **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 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: Accurately weigh the amount of *Salix* Species Bark Dry Extract calculated to contain about 30 mg of salicin, 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 Dry Extract 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 Dry Extract taken to prepare the *Sample solution* (mg)

Calculate the percentage of the labeled content of salicin in the portion of *Salix* Species Bark Dry Extract taken:

$$\text{Result} = (P/L) \times 100$$

P = percentage of salicylates as determined above
L = labeled content of salicin

Acceptance criteria: 90.0%–110.0% of the labeled amount of salicylates calculated as salicin on the anhydrous basis

CONTAMINANTS

- **Articles of Botanical Origin** ⟨**561**⟩**,** *Pesticide Residue Analysis***:** Meets the requirements
- **Botanical Extracts** ⟨**565**⟩**,** *Preparations***,** *General Pharmacopeial Requirements***,** *Residual Solvents***:** Meets the requirements
- **Microbial Enumeration Tests** ⟨**2021**⟩**:** The total aerobic bacterial count does not exceed 10^4 cfu/g, and total combined yeasts and molds 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 and Limit of Free Salicin**[2]

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: Weigh the amount of *Salix* Species Bark Dry Extract calculated to contain about 15 mg of salicin, transfer to a 50-mL volumetric flask, add 25 mL of methanol, and sonicate for 5 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* 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.

- **Water Determination** ⟨**921**⟩**,** *Method I, Method Ia***:** NMT 5.0%
- **Articles of Botanical Origin** ⟨**561**⟩**,** *Methods of Analysis***,** *Total Ash*

Sample: 2.0 g of *Salix* Species Bark Dry Extract
Acceptance criteria: NMT 5.0%

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 from which the article was prepared. The label also indicates the content of salicin, the solvent used in extract preparation, and the ratio of the starting crude plant material to dry extract. It meets the labeling requirements of *Botanical Extracts* ⟨565⟩.
- **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*)

[2] Elevated free salicin content may indicate prehydrolysis or fortification with extraneous salicin.