

## 0069 - Willow Bark for Salicins by HPLC

**Botanical Name:** *Salix alba L. and other species*

**Common Names:** White willow bark

**Parts of Plant Used:** Inner bark

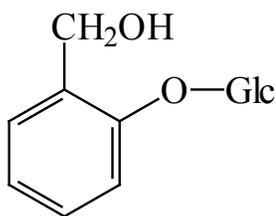
**Uses:** For pain relief and fever reduction; as an anti-inflammatory agent.

### Modes of Action:

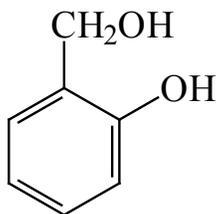
Willow bark is known to contain salicin-type compounds which are responsible for its bioactivity. In the human body, salicin decomposes to salicylic acid by oxidation of salicyl alcohol, which is formed upon intestinal hydrolysis of salicin. Salicylic acid is the final bioactive compound.

### Chemical Markers:

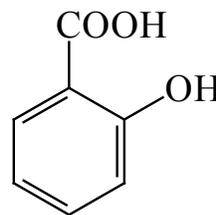
The chemical components of several willow species have been studied thoroughly. The barks were found to contain different amounts of phenolic compounds, depending on the species, the source, the collecting time, and the age of the trees. The identified phenolics include proanthocyanidins (monomers, dimers, trimers), flavonoids (rutin, naringenin-7-glucoside, luteolin-7-glucoside, quercitrin, naringenin-5-glucoside, quercetin), glycosides of phenols and phenolic acids (salicin, fragilin, tremuloidin, salicortin, tremulacin, populin).<sup>1,2</sup> Currently, salicin is used as marker compound for quality control of willow bark extract, especially white willow bark extract.



Salicin



Salicyl alcohol



Salicylic acid

## Methods of Analysis

Although willow bark has been known for its anti-inflammatory activity for a long time, only a few analytical methods have been applied to analyze the phenolic compounds in it. Currently, HPLC is the most widely used method for analysis of salicin in willow bark extract.

### Method 1:

The HPLC method of Meier et al.<sup>3</sup> was used.

#### *Sample Preparation:*

Extract 200 to 500 mg of dried material in a clipping homogeniser with 25 and then 40 mL of methanol, wash and then with 20 mL methanol. Evaporate the solution to dryness at less than 40°C. Dissolve the residue in 10 mL of methanol–water (7:2).

#### *Chromatography:*

Column: Spherisorb ODS II, 5 µm, 100 × 4 mm.

Mobile phase: Solvent A = water–tetrahydrofuran–phosphoric acid (97.7:1.8:0.5), solvent B = methanol.

Gradient:

Time (minutes)	%A	%B
0	100	0
5	100	0
10	85	15
20	70	30
30	65	35
40	50	50
45	50	50

Flow rate: 1 mL/minute

Injection volume: 10 µL

Detection wavelength: 270 nm

#### *Validation Data:*

Not available

### Method 2:

In the method of Luo et al.,<sup>4</sup> the salicins are first converted to salicyl alcohol by enzymes and then analyzed by HPLC.

#### *Sample Preparation:*

Extract 1 g of powdered sample with 20 mL of extraction buffer (0.01 M aqueous potassium phosphate containing 0.02% sodium dodecyl sulfate, final pH 5.0) in an 80°C water bath for 30 minutes. Centrifuge for 10 minutes at 2000 × g and dilute 0.5 mL of the supernate to 10 mL with extraction buffer. Pipet 1 mL of diluted extract into a 10-mL test tube and add 1 mL of beta-glucosidase solution (2 mg/mL). Incubate the mixture at 37°C for 40 minutes.

#### *Chromatography:*

Column: Phenomenex Prodigy ODS-3, 5 µm, 250 × 4.6 mm.

Mobile phase: Solvent A = water (0.02 M KH<sub>2</sub>PO<sub>4</sub> with 0.014% tetrabutylammonium hydrogen sulfate), solvent B = acetonitrile; isocratic 84%A and 16%B.

Injection volume: 100  $\mu$ L

Detection: Fluorescence detector with  $\lambda_{ex}$  = 275 nm and  $\lambda_{em}$  = 300 nm for the first 12 minutes, and then switch to  $\lambda_{ex}$  = 295 nm and  $\lambda_{em}$  = 400 nm.

Flow rate: 1 mL/minute for the first 12 minutes, then increase to 1.5 mL/minute.

#### **Validation Data:**

Linearity: 8 to 800 mcg/g for salicin and 2 to 160 mcg/g for salicylic acid.

Accuracy: More than 96% recovery with a correlation coefficient of 0.9998 for salicin and more than 89% recovery with a correlation coefficient of 0.9999 for salicylic acid; three types of willow bark were tested.

Precision: Not specified

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ: LODs = 4.0 mcg/g for salicin and 0.05 mcg/g for salicylic acid; LOQs = 20 and 1 mcg/g for salicin and salicylic acid, respectively.

#### **Method 3:**

The unpublished method of Mingfu Wang was used.

#### **Sample Preparation:**

Weigh the appropriate amount of material (150 mg for 15% white willow extract) into a 50-mL volumetric flask. Add about 35 mL of 20% methanol and sonicate for 20 minutes and shake for 20 minutes. Allow the flask to cool to room temperature and fill to volume with 20% methanol.

#### **Chromatography:**

Column: Phenomenex Luna C18 (2), 5  $\mu$ m, 4.6  $\times$  250 mm.

Mobile phase: Solvent A = 1% acetic acid solution, solvent B = acetonitrile.

Gradient: 8%B to 32%B in 20 minutes.

Flow rate: 0.8 mL/minute

Injection volume: 10  $\mu$ L

Detection wavelength: 271 nm

#### **References:**

1. Balbaa SI, Khafagy SM, Haggag MY, et al. Phytochemical study of certain *Salix* species cultivated in Egypt. *Egypt J Pharm Sci.* 1979;20(1-4):153-64.
2. Bruneton J. *Pharmacognosy, phytochemistry, medicinal plants.* Paris: Lavoisier Publishing; 1995:222-3.
3. Meier B, Julkunen-Tiitto R, Tahvanainen J, et al. Comparative high-performance liquid chromatographic determination of phenolic glucosides in Salicaceae species. *J Chromatogr A.* 1988;442:175-86.
4. Luo W, Ang CY, Schmitt T, et al. Determination of salicin and related compounds in botanical dietary supplements by liquid chromatography with fluorescence detection. *J AOAC Int.* 1998;81:757-62