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Short communication

Capillary electrophoretic analysis of salicin in *Salix* spp.

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Abstract

Salicin is a phenolic glycoside in willow bark which exhibits analgesic effects. Its occurrence in willow (*Salix*) species is the major reason willow bark and its extracts are popular products in the nutritional supplement market. Previously, HPLC has been the standard method of analysis of quantifying salicin in *Salix*. We describe a new method of analysis using capillary electrophoresis (CE) that is faster, relatively simple and highly reproducible. Two different species of willow, *Salix alba* and *Salix scouleriana*, are analyzed for salicin by CE, using 20 m*M* glycine (pH 8.8), in uncoated capillaries at 25 kV and 25°C, and UV detection at 215 nm. © 1997 Elsevier Science B.V.

Keywords: *Salix* spp.; Salicin; Glycosides

1. Introduction

Willow bark contains a variety of glycosides including salicin, 2-(hydroxymethyl)phenyl-β-D-glucopyranoside, which exhibits an analgesic effect [1]. Although the total phenolic glycoside content ranges from 2 to 11%, the bark contains various smaller amounts of salicin, depending upon species diversity, harvest times, seasonal variations, gender and age of the plant, and the portion of the bark harvested [2]. Furthermore, alkaline hydrolysis converts many of the phenolic glycosides, such as salicortin, fragilin and tremulacin, into salicin [3,4]. A number of reports about the salicin and total glycoside content in various species have appeared, using various analytical methods such as TLC [5–7] and HPLC [2].

However, to this point in time, no reports have appeared that apply capillary electrophoresis (CE) to the analysis of salicin. We report a new, rapid method for the quantitative analysis of salicin and apply it to three samples of willow bark. In addition to salicin content, differences in species can be seen observed, based upon the diversity of substances present in the samples.

2. Experimental

2.1. Apparatus

Separations were performed using a Spectrophoresis 1000 CE system, containing an autosampler, capillary temperature control, and a rapid scan UV–Vis detector (ThermoSeparations Products, San Diego, CA, USA). The instrument was interfaced to a Pentium 90 computer running PC1000 ThermoSeparations Products software, version 3.0.

Uncoated, open, 50-μm diameter, silica capillaries were purchases from Polymicro Technologies (Phoenix, AZ, USA) and cut to 75 cm.

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2.2. Reagents and samples

Glycine (free base) and salicin standard from were obtained from Sigma (St. Louis, MO, USA). Water was distilled and deionized before preparing buffers and samples. Three authenticated willow bark samples were obtained from Mark Lange of Industrial Laboratories (Denver, CO, USA). Two samples of *Salix alba* (each from different sources) and a sample of *Salix scouleriana* were analyzed.

2.3. Electrophoresis conditions

Silica capillaries were preconditioned by washing with 1 *M* NaOH for 15 min at 60°C, followed by 0.1 *M* NaOH for 15 min at 60°C, and distilled, deionized water for 15 min at 25°C. Prior to each injection, the capillary was washed with two column volumes of 0.1 *M* NaOH, followed by four column volumes of fresh running buffer, which consisted of 10 m*M* glycine adjusted to pH 8.8 with NaOH. Glycine buffers offered better resolution of salicin from its neighboring peaks than other buffers such as phosphate or borate at pH values from 8.0 to 11.0. Samples were introduced by hydrodynamic injection for 1 s. Separations were run at 25 kV and 25°C, and monitored via UV absorption at 215 nm.

2.4. Procedure

One-gram samples of each willow bark sample (weighed to 0.001 g) were extracted for 1 h in 100 ml of boiling water under reflux. After cooling, each sample was filtered through Whatman No. 4 filter paper, and then through a 0.45-μm filter. One volume of the resulting amber-colored solution was mixed with one volume of 20 m*M* glycine buffer, previously adjusted to pH 9.0 with NaOH. (Dilution of the buffer resulted in a final pH of 8.8.) This solution was placed directly into an injection vial and analyzed by CE.

Standard solutions of salicin were prepared by dissolving 0.100 g of salicin standard in 100 ml of running buffer (10 m*M* glycine, pH 8.8), then diluting this solution with more running buffer and filtering to obtain various concentrations ranging from 0 to 100 μg/ml salicin.

3. Results

3.1. Electropherograms

As seen in Fig. 1, the salicin peak appears at 4.8 min, leading a complicated array of many other substances, which continue to emerge for up to 60 min under these conditions. The rapid migration of salicin compared to the other UV-absorbing components allows its successful resolution in a relatively short time.

3.2. Calibration and sensitivity

Standard curves derived from integrated areas of pure salicin yielded an excellent linear fit (five points; slope=150, intercept=0.001, standard error of coefficient=1.13, r^2=0.9997). Internal addition also yielded excellent agreement with the external standard curve. As seen from the data in Table 1, spike recovery in various samples ranged from 97 to 106%, with an average of 98.5%. The average migration time for salicin was 4.91 min, with a relative standard deviation of 0.83%.

The relative standard deviation of peak area for the salicin peaks during calibration and sample analyses was approximately 6.5%. However, when sodium benzoate (migration time 11.2 min, at 50 μg/ml) was added as an internal standard to correct for injection volume, the relative standard deviation for peak area was reduced to 2.6%. The limit of detection observed for this method was 75 ppm salicin, determined using pure standard solutions.

3.3. Analytical results

According to our analysis by CE, both samples of *Salix alba* contained less than 1% (w/w) salicin. One sample contained 0.31% (±0.02%) and the other 0.78% (±0.03%). *Salix scouleriana* contained a much higher level of 1.39% (±0.09%).

4. Discussion

CE is capable of rapidly analyzing salicin from willow bark: in less than 5 min. This is significantly faster than HPLC methods, which usually exhibit

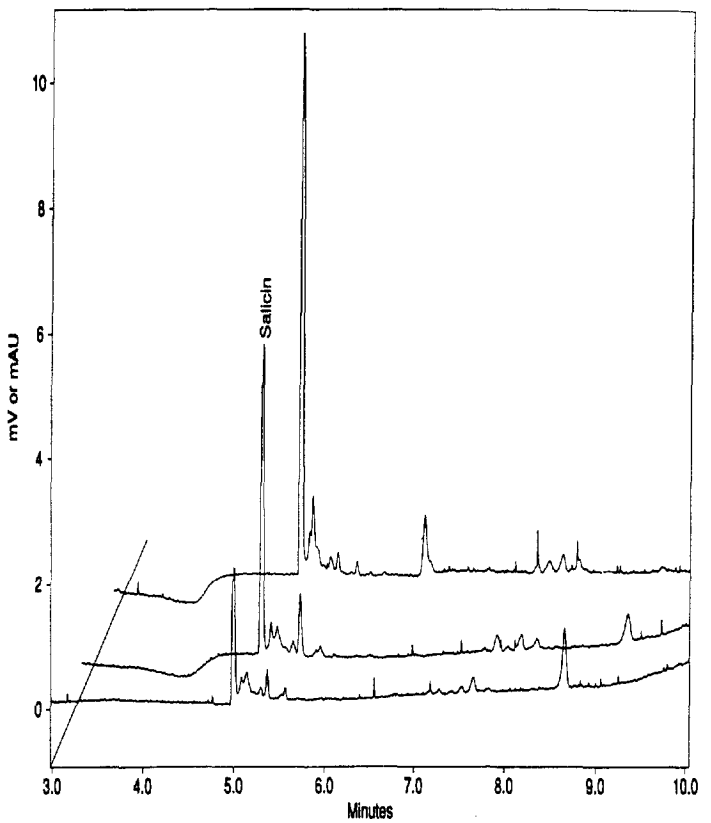

Fig. 1. Electropherograms of three samples of willow bark. The two lower traces are of *Salix alba* and the upper trace is of *Salix scouleriana*. The running buffer was 10 m*M* glycine, pH 8.8; hydrodynamic injection for 1 s; separation at +25 kV, 25°C, UV detection at 215 nm.

retention times of 15–45 min [4]. The ease of sample preparation is also quite simple, utilizing a hot-water extract. A 100-fold volume of water was sufficient to quantitatively extract the naturally occurring salicin, as evidenced by lack of detectable salicin remaining in subsequent extractions. This extraction method is selective for the naturally occurring salicin, as opposed to 'total salicin', which can be determined if the powdered bark sample is subjected to alkaline hydrolysis before analysis.

Table 1
Statistical data for salicin analyses with internal standard

Salicin concentration	2.00 μg/ml
Salicin spike conc.	0.400 μg/ml
Spike recovered	0.394 μg/ml
Percent recovery	98.5%
Replicates (*n*)	12
Average migration time	4.91 min
R.S.D. (migration time)	0.83%
R.S.D. (salicin conc.)	2.60%

CE conditions: 10 m*M* glycine, pH 8.8, 25 kV, 25°C, UV detection at 215 nm.

This work represents the first application of CE to the analysis of salicin from willow bark. The results of our work agree closely with values previously reported [4], namely that samples of *Salix alba* routinely contain 0.1–1.0% (w/w) in air-dried bark. When grown under different conditions, samples of bark from trees of the same species often differ in concentrations of their phenolic glycosides, which is the case in these two samples of *Salix alba*.

On the other hand, *Salix scouleriana* is known to have significantly higher amounts of salicin than *Salix alba*, which is corroborated by our data. It is

also noteworthy, that the fingerprint electropherogram in the region between 5 and 10 min exhibits variation between the *S. alba* and *S. scouleriana* species. For example, the unidentified peak(s) at 6.5 min are present in *S. scouleriana*, but missing in *S. alba* (see Fig. 1). Such markers may be useful in distinguishing between species of dried, ground plants as supplied in nutritional supplements.

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